

nature

May 15, 1975

Are the art treasures and the overseas students safe?

LAST week it was Mr Reg Prentice's turn, as Secretary of State for Education and Science, to appear at the House of Commons Select Committee's hearing on the financing of scientific research in British Universities. To his sympathetic questioners he declared that the universities were marginally more able, twelve months ago, to bear cuts in their funding than were most other parts of the educational system. There had been a generosity in the staff-student ratio which could bear a little squeezing, but now that had been done, and he did not think one could any longer generalise on the ability of universities to stand further onslaughts. But perhaps some well-endowed establishments might sell off a few of their oil-paintings to tide them over the crisis. The British Antique Dealers' Association pronounced the idea "morally reprehensible".

If we are down to dusting off the art treasures, then we are also at the level at which some other appurtenances of university life are vulnerable. The committee has already had a nose around unproductive research without much in the way of enthusiastic response from its witnesses. Where it does seem to be generating some momentum of its own is on the issue of postgraduate students from overseas. Thus Mr Norman Tebbit asked Mr Prentice whether overseas students were not occupying too much of the "well found" floor space supported by the University Grants Committee (UGC) and hence the taxpayer. Mr Prentice gave the standard deadbat response that there were long-term diplomatic and commercial benefits to having overseas students here.

It was clear that there is a view on the committee not only that overseas students could be charged rather more for their time here but also that there are some cases where the benefits of having overseas students are mainly benefits to the sending countries. This view is helped along by two observations: first that there are departments, particularly in the applied sciences, where overseas postgraduates are in the majority and, second, that although nobody is questioning the concept of aid to developing countries through the provision of university education, a growing number of postgraduate places is being taken up by students from the developed world.

The concern is a reasonable one within many people's concept of what a university should be, and deserves serious attention. In the five years from 1968 to 1973 the number of those obtaining higher degrees (in all subjects) has grown steadily from around 10,000 to around 14,000 a year. (Roughly 40% of these degrees are doctorates.) While the number of Commonwealth higher-degree recipients has remained static at about 1,500, the numbers from other countries has doubled from 1,000 to 2,000. In 1973, 27% of all recipients of higher degrees were from overseas—in pure science 22%, in applied science 36%. About 60% of overseas students returned home after obtaining their degree. In the same year 376 first-degree

British graduates went overseas for further education; this figure has declined from 538 in 1970. Most recent figures show that overseas students now account for 33% of post-graduates and applied sciences probably over 40%.

Britain gives more than she takes in higher education, and fairly clearly she has been giving more to students from developed countries in recent years. Is there a case for any control of numbers? The reasons normally advanced against the suggestion are not the most compelling.

Although it is true that the training of students here generates a lot of goodwill in academic, industrial and governmental life around the world, it would be difficult to demonstrate that this creates much in the way of hard returns beyond providing professors with a choice of venue for their sabbaticals. If postgraduate training increases sophistication in students, it must be assumed that they are unlikely to be swayed in the purchase of, say, some piece of equipment by the fact that they went to university in the country in which the makers are situated. And if a piece of equipment being considered is one with which they had experience when in Britain, it is entirely possible that it was imported into Britain or did not prove satisfactory (or both).

The diplomatic profit argument is no easier to sustain. Armies lead by Sandhurst-trained officers have been known to act in other than Britain's interests. Is there any evidence that London-trained ministers of trade, technology, communications, oil and so on look any more kindly on the country in which they obtained their postgraduate education? Any Harvard-trained minister in the British government who started favouring US business would soon be out on his ear.

There are two much better arguments for encouraging the flow of overseas students—one idealistic, one chauvinistic. On the idealistic plane, Britain supports universities because they are places of learning, and learning, even in the applied sciences, knows few frontiers. True, the man with the key patent will profit himself and his own country first, but the benefits very quickly flow to the users as well as the inventor. And the inventor will have had to dip into a truly international pool of knowledge. Thus talk of great new British inventions, so beloved of newspapers, is often meaningless. The more the international character of learning is stimulated by the interchange of people, the more broadly and effectively does the academic life operate. (But fewer Britons are going abroad for postgraduate education.)

On the chauvinistic level, every postgraduate who comes to Britain is potentially capable of making a significant intellectual contribution even within a three-year period—and many do. It is often forgotten that some do their best work when still a student, and this work is then immediately available in the British community. Besides which, many stay for the rest of their lives. □



Famine revisited

Recent reports from Ethiopia indicate that famine has again struck the pastoral population of the south. A year ago an article in Nature made the case that the collection of objective information in droughts is of the first importance in planning relief operations. The London Technical Group has undertaken the design and conduct of such surveys in Ethiopia and elsewhere. In this article Julius Holt, John Seaman and John Rivers of that organisation discuss their observations made on the pastoralists and farmers of Harerge Province, South-east Ethiopia, in mid-1974 after the first wave of drought. They comment on the failure of relief efforts to prevent death and disruption when the rains again failed.

POPULATIONS do not suddenly starve; but starving populations becoming sudden and dramatic news. The process leading to famine after a natural catastrophe is prolonged and complex and occurs against a background of endemic malnutrition and poverty. By the time a famine is internationally recognised it is already at or past its peak, and relief aid does not usually begin to arrive in significant quantities until the starvation crisis is over and the majority of famine deaths have occurred. Emergency aid is, moreover, disproportionately costly, and economic considerations therefore support the humanitarian reasons for investing in timely and informative surveys to warn of developing famines and to guide effectively in their relief.

A year ago, workers from the London Technical Group and the Medical Research Council Dunn Nutrition Unit described in *Nature* their experiences in the drought-affected areas of north-eastern Ethiopia, pointing out the need to develop standardised survey techniques both for rapid famine assessment and for monitoring the situation during the danger period. By that time the focus of the drought was no longer in the agricultural highlands of the north-east but had shifted to predominantly pastoral regions, particularly the vast rangelands of Harerge Province in South-east Ethiopia. Some reports of starvation emanated from that area as early as the end of 1973.

The importance of rapidly gained but systematic information had won increasing recognition among relief agencies. Members of the London Technical Group were invited by the Ethiopian Relief and Rehabilitation Commission to design and direct a survey of Harerge from May to July 1974, and the project was supported by UNICEF, USAID, Oxfam and Christian Aid.

The purpose of the survey was twofold: the most urgent objective was to discover as rapidly as possible the nutritional condition of the population, but it was also hoped that the information gained would form the basis of a longer-term surveillance scheme. The problems presented were not inconsiderable. The geography of the region to be surveyed was daunting, for Harerge, which shares an extensive border with northern Somalia, is larger in area than England but has an extremely poor road system. A highland ridge, supporting an agricultural population of some 2.2 million, cuts into a semi-desert lowland ('Issa and Ogaden) which is seven times greater in area, with a population in normal times of up to 0.5 million pastoralists. There was a profound lack of baseline data on the area, which

made it impossible to relate the survey sample accurately to the population distribution and thus made it necessary to include a large retrospective element in assessment.

The logistical difficulties were greatly reduced by the provision of three helicopters by USAID which, with some ground transport, allowed survey teams to make overnight stays at 65 sample sites within a month. But the difficulties inherent in the lack of baseline data remained largely intractable, especially since the effects of the drought were at the time of survey less dramatically obvious than some had expected.

The survey of the nutritional status of more than 1,000 children showed that both pastoral and agricultural populations were generally in a poor nutritional state, but severe protein-energy malnutrition was exceedingly rare. During the preceding twelve months death rates reported to us for children, particularly those of pastoral groups, had, however, been far higher than any reasonable estimate of mortality in a developing country would lead one to regard as normal, and the current demographic picture supported these reports. It was clearly not possible to be certain of the causes of death, but we were satisfied that no epidemic had occurred, and there seemed no reason to doubt the statements of the people that the chief cause had been malnutrition.

Experience elsewhere had led us to suspect that we might indeed be faced with a population which had suffered a tragedy but which at the time of the survey would not exhibit signs of extreme undernutrition. Much of the enquiry was therefore directed to a rapid attempt to define the impact of the drought on food availability for the near future. The Ethiopian Ministry of Agriculture had reported patches of poor harvest in the highland areas of the province, with some serious losses among the cultivators of the lower hills. Local people reported extremely high losses of livestock (cattle, sheep, goats and camels) and systematic observations during the survey of livestock held by pastoral groups showed that herds and flocks held were at about the number which had been reported as the minimum necessary for survival in the area. Before the main 'Gu' (March-May) rains, which had occurred just before the survey, these had been in poor condition and their lowered milk production could well have caused much of the mortality.

But the problem had been compounded by the fact that grain prices had risen steeply, and the survey found that rises were particularly extreme in the Ogaden where grain had been un-

available in some markets at times during the year. The significance of this is that the pastoralists live not only on the produce of their animals but also by selling them in order to buy grain. At the time of the survey the combination of livestock losses, the fall in the cash value of animals and the rise in the cost of grain had reduced the effective wealth of many groups to less than 20% of the level of the previous year and to under 10% of that of times before the drought. During the height of the drought some animals had been unsaleable and herders had been forced to eat animals they were no longer able to keep alive. The nutritional implications of this are considerable: for example, a heifer sold for grain would normally buy at least 8 times as much food (in calories) as are available from its meat.

The survey showed that the physiological reserves of this population were low and that their economic reserves had fallen to a point which in many instances was not far from that of no return. In short, this was a highly precarious nutritional situation. But the implications for relief were problematic. Grain is the basic currency of relief for pastoral as well as agricultural populations. But the logistics and administrative difficulties of distributing grain to a mobile population have never been successfully solved. The scheme actually adopted relies on the encampment of groups round administrative centres, thus simplifying the transport of grain. The result is large congregations of women and children together with some aged people; the men continue to move with the animals. There are three inherent dangers in this: first, the threat of long-term disruption in the social lives of families and groups; second, the risk of epidemic disease in such large congregations; third, the possibility that groups will not arrive in centres in time to avoid some mortality. The temptation to avoid stress for whole groups, including the men, to become entirely dependent in the long-term on grain hand-outs is of concern to the authorities, and has important implications for the future of the pastoral economy in an area where agriculture will not be a viable alternative at least for many years to come.

At the time of the survey we concluded that a further failure of the short rains in September–October 1974 was likely to be catastrophic for the pastoralists, whereas normal rains would allow them to take a vital step in strengthening their self-sufficiency. The recommendations, therefore, were that although resources should be husbanded for a major relief operation in the event of rain failure—by stock-piling grain and making provision for the large amount of transport which would be

required—the other urgent priority at that time (July 1974) was the institution of a surveillance programme to monitor changes in the situation. Unfortunately the kind of sustained field-surveillance originally envisaged did not materialise. Meanwhile, a limited relief programme continued for the relatively small numbers of destitute people who were then clustered around centres.

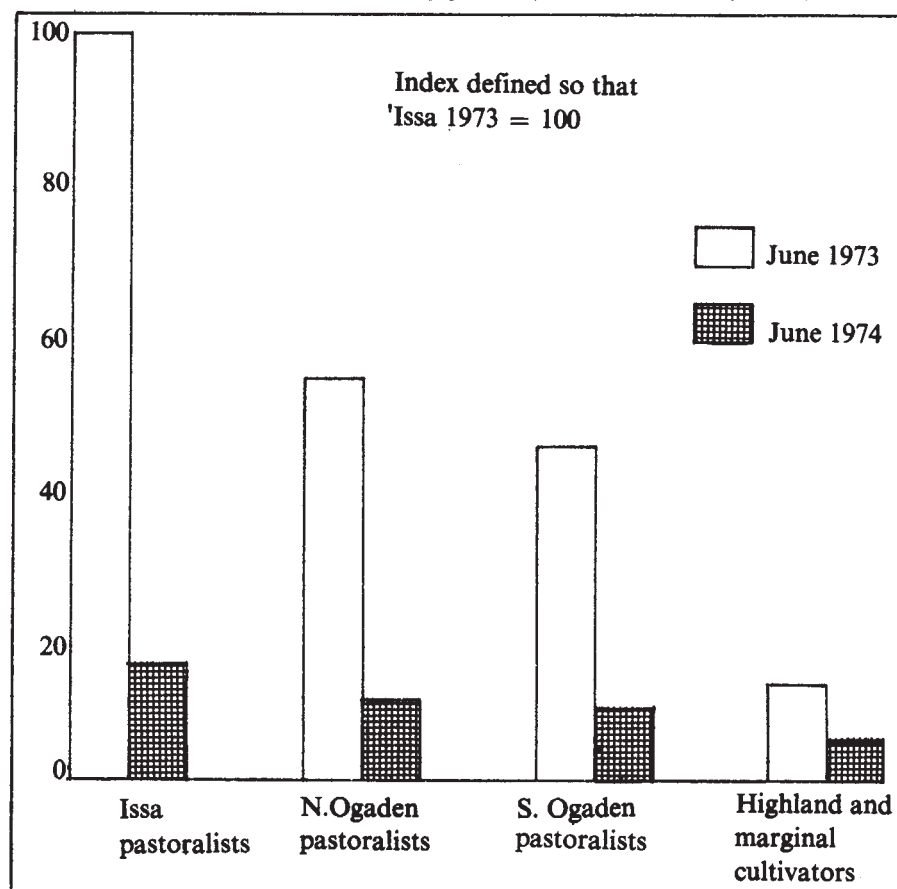
In the event the autumn rains were sparse. Reports reaching us from the Ogaden in the first part of 1975 spoke of a deteriorating situation with numbers around relief centres (mostly women and children) growing to 50,000. A recent report from Oxfam cited a peak death rate in this group of 150 children a day, which, in spite of the efforts of medical teams, had not fallen below 50 a day by early April. Such numbers of people in such condition offer a disturbing indication that the linked problems of surveillance and effective relief there are far from overcome.

Most estimates of food availability in developing countries depend on ratios of estimates of crop yield and spoilage to estimates of population, and are subject to large errors. In particular, the sensitivity of such ratios as indicators of change is poor. Prediction of famine is only possible at the most general level by such techniques, and it is impossible to pick up the smaller

local shifts between hardship and disaster. On the other hand, a comprehensive assessment of a famine situation has been shown to be possible using the kind of nutritional and socioeconomic measurements that formed the basis of the survey described here, with the special advantage that, provided care is taken with the sampling technique, the accuracy of the system is sufficient to indicate relatively small changes in the nutritional status of local populations.

Baseline surveys and the organisation of repeat surveillance should be considered a priority in those areas of the developing world which are now identifiably prone to extreme localised food shortages. This would not constitute a large diversion of resources. For instance, in spite of its size the Harerge survey cost, helicopters apart, less than £10,000. In subsequent surveillance of such areas the body of the work does not need highly trained personnel or a high capital investment in sophisticated equipment. The use of expensive air transport tends to be the price paid for belated reactions to an emergency. In any of the recent African droughts an effective field-surveillance programme could have been mounted for less than 1% of the cost of the total relief programme, and could have helped to ensure that for the first time aid arrived before people died of starvation. □

Purchasing power of animal herds in grain equivalents.



AN article by C. L. Crawford (*Nature*, March 20), stressed in vigorous terms the well recognised concept that the best available means of secondary prevention of leprosy lies in the regular and sustained treatment of leprosy sufferers with the cheap and effective mycobacteriostatic drug, dapsone. We agree, and have said the same, many times.

The well-nigh insuperable difficulties facing the World Health Organisation and voluntary agencies, in trying to implement a strategy on which they are now generally agreed, are concerned with factors to which Crawford seems to pay little attention in his article. These factors are, on the one hand, the social aspects of leprosy, their effect on government policy and priorities, and patient attitudes to leprosy, and, on the other, the inadequacy of diagnostic and curative facilities in precisely those countries where leprosy constitutes a major health problem. Add to this the long duration of treatment advised and the need for regularity of treatment—if deformity is to be prevented and the risk of drug resistance minimised—and the world situation can be seen in its stark reality, which is reflected in successive reports from the World Health Organisation and its Expert Committee.

Those of us who have been concerned for upwards of 40 years with mass treatment schemes for leprosy and ambulatory treatment/control measures integrated into the general urban-rural health services cannot but agree that “sulphones provide a cheap and practical form of treatment and also prevent transmission of leprosy”. A recent editorial in *Leprosy Review* says the same thing: “The maximal extension of sulphone treatment, and a skilled sustained campaign of leprosy education are priorities for us all.” The problem is to discover large scale schemes, adequately documented and supervised, in which this thesis can be conclusively demonstrated. An oversimplification of his case, illustrated by atypical populations with “a small leprosy problem” and highly selective official estimates of incidence rates, may not convince the cautious epidemiologist.

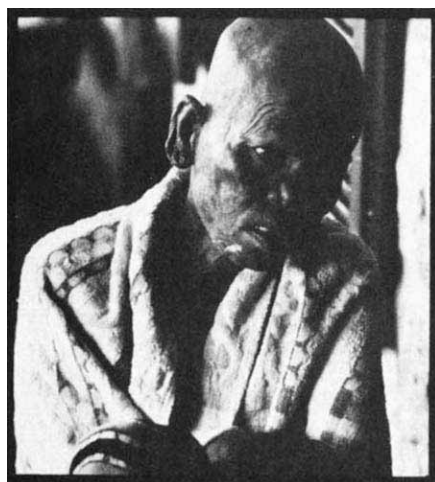
It would be a salutary exercise to expose the deficiencies in Crawford's article: “the mode of transmission and the incubation period are unknown”; “there is no . . . vector to spread the disease”; “only one in ten of the exposed population, at the most, acquire the disease”; leprosy declined in Norway “by a policy of segregation”; “once eradicated from any country, it has never returned”; “no attempt has been made to obtain information about incidence rates”; “accurate figures of leprosy are available . . . in Rumania,

Cyprus and Libya”.

From recent personal observations, we consider that the conclusions drawn by Crawford regarding the situation in some of the countries he refers to are not in accordance with the facts; on the contrary, the total prevalence rates (comprising the accumulated back-log of high incidence rates associated with a virtual breakdown of rural health services) are much higher than they were 15 or 20 years ago. This does not, of course, invalidate his main thesis that regular and adequate sulphone

What kind of strategy for leprosy?

By S. G. Browne, Director of the Leprosy Study Centre, and T. F. Davey, of Leprosy Review.



therapy could eventually result in a reduction of incidence rates, but it does emphasise the realities of today's world and the intractability of the problems of the control of transmissible diseases, among which leprosy figures along with malaria, tuberculosis, trypanosomiasis, onchocerciasis, schistosomiasis and the rest.

Crawford asserts that “drug resistance is very rare”. It was, but it is not now, and the recent figures coming from countries where the problem has been adequately investigated are very disturbing, to say the least.

“In many (countries) the leprosarium remains the only place where treatment can be obtained”. This statement is hopelessly out-of-date, as is the incredible assertion that, of the £2 million raised annually by voluntary agencies in Europe for leprosy work, most is still spent on institutional care. More than 900,000 leprosy sufferers are being treated at the moment through programmes assisted financially by these maligned voluntary agencies, and the

vast majority of these patients are being treated in ambulatory schemes; in addition, about £250,000 a year is being devoted to leprosy research. About a third of all leprosy patients now receiving any treatment at all are getting it through these same voluntary organisations. If such mis-statements are allowed to pass uncorrected, a grave disservice will be done to the cause of truth and to the leprosy sufferer.

As for prophylaxis, to judge by the tempo of much cooperative research based on the availability of relatively enormous quantities of *M. leprae* obtained from experimentally infected armadillos, a specific vaccine should be produced in the not-too-distant future, together with a specific skin test. Chemoprophylaxis (with dapsone) has been shown to be effective, as Crawford points out, but here again the practical objections to large scale implementation are over-riding. Countries where such a measure might be justifiable on general grounds lack both the medical infrastructure and the finance, not to mention the cooperation of the population necessary over a prolonged period (possibly 3–5 years) during which the regular ingestion of a toxic drug by mouth (dapsone) or by intramuscular injection (acedapsone every 75 days) would be necessary.

Ministries of Health the world over are facing the problem of a chronic crippling disease, which, ideally and considered in isolation, might require a special service with vast resources—for diagnosis, laboratory cover, prolonged treatment (with a drug that is cheap, admittedly, but whose real cost is greatly enhanced by the expense of getting it to the patients who need it), preventive physiotherapy, provision of protective footwear, not to mention rehabilitation facilities. The social and medical dangers of over-emphasising one of the endemic diseases lie in creating or perpetuating the idea of ‘uniqueness’ and ‘difference’ and hence encouraging the stigma attached to the disease.

If treatment of leprosy could be rapid as well as cheap, unaccompanied by severe episodes of reaction as well as effective, capable of bacillary clearance as of mycobacteriostasis, then Crawford's fundamental thesis would be gladly accepted by governments. Meanwhile, inadequately staffed and inadequately financed health services in many countries in the Third World, while awaiting a more effective strategy of control based on primary prevention of leprosy, are striving with varying degrees of enthusiasm and success to put into practice the principles of secondary prevention based on the availability of a cheap and reasonably effective drug, dapsone. □

international news

DURING the late 1960s, two scientists employed by the Atomic Energy Commission (AEC), John Gofman and Arthur Tamplin, launched a public attack on the AEC's regulations governing permissible standards of exposure to ionising radiation. They contended that the regulations were too lax because people exposed to radiation at the permitted levels would run an unacceptable risk of developing cancer. The AEC responded in kind with a vigorous public defence of its regulations. But, according to Gofman and Tamplin, it also responded with a private attack on its two troublesome scientists—it greatly reduced their laboratory budgets and staffs.

That episode highlights a problem which has been cropping up with increasing frequency, namely, how can scientists who feel a moral obligation to speak out on particular issues do so when such actions place them in direct conflict with their employers or with other powerful vested interests? And that particular episode also provided a catalyst for the establishment by the American Association for the Advancement of Science of a committee to investigate such conflicts.

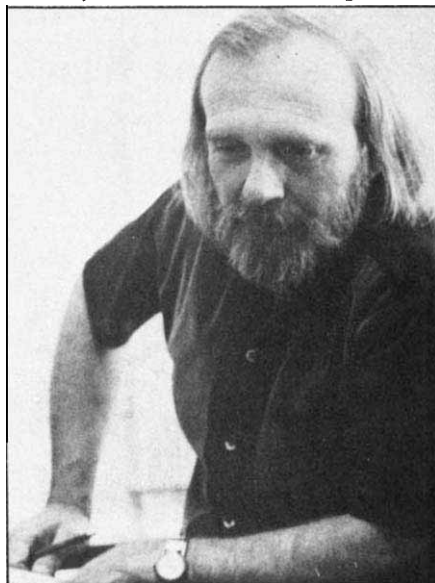
The committee's report finally appeared last week after an inordinately long gestation period of 4½ years. Written for the committee by John T. Edsall, a Harvard biologist, it stands out as a thoughtful and eloquent statement on academic freedom and scientific responsibility—a field which has all too frequently been a subject of much academic claptrap.

First, Edsall discusses some of the moral and ethical concerns which have arisen among scientists themselves over a few areas of research, and which could result in restrictions on academic freedom. Noting that "the basic function of the scientific community is the advancement of knowledge", he suggests that some scientists "might well believe that nothing should be off limits as a possible subject for basic research, as distinct from applied science and technology". But he reckons that "there may be strong reasons for declaring certain areas of research off limits, or at least for imposing severe restrictions on them".

Some types of experiment on human subjects, for example, "raise serious questions" and should be carried out only according to strong ethical and practical guidelines which may inevitably rule out some of the more dan-

Scientific freedom and responsibility

by Colin Norman, Washington



Tamplin: troublesome scientist

gerous activities. Moreover, the recent call for a temporary halt in some types of genetic manipulation experiments was "a landmark in the assumption of scientific responsibility by scientists themselves for the possible dangerous consequences of their work".

But, on more controversial ground, Edsall offers a strong defence of the legitimacy of research into the role of genetic factors in human intelligence. It has been argued that such research should be declared out of bounds because it could exacerbate racial prejudices. But Edsall strongly supports "the view that inquiry into genetic differences between racial groups is a thoroughly legitimate field of research, and we condemn the emotional attacks that have been made against such persons as Arthur Jensen and R. J. Herrnstein, because of their views concerning the importance of genetic factors in human intelligence. Their views are, of course, open to critical debate and challenge on scientific grounds, as long as the debate is conducted on an objective scientific level".

The problem, however, is that once such views enter the political arena, the debate automatically leaves the "objective scientific level", and the real

question is what responsibility scientists should have for the uses to which their research findings are put. On the particular question of racial differences in intelligence, Edsall offers only the suggestion that "it is wise for investigators to state their results cautiously and modestly, to specify clearly the limits of accuracy, and to refrain from sweeping claims based on highly specialised researches".

As for conflicts which arise when scientists blow the whistle on their employers or make public statements on scientific issues which place them in conflict with vested interests, there are many examples of the subjection of scientists to professional harassment for trying to defend what they see as the public interest. The case of Gofman and Tamplin is one example. Another is the case of three engineers working on San Francisco's Bay Area Rapid Transit System. In 1972, they were summarily dismissed, allegedly for making statements about defects in the train control system—statements which later turned out to be correct.

There have also been occasions when scientists working for industrial concerns have sided with the company in suppressing data damaging to their employers' operations or in resisting changes which would result in more rigorous health and safety standards.

In such instances, Edsall argues that professional societies should play a much more active role in defending the interests of their members or in adjudicating in disputes where issues of scientific freedom are involved. The societies "can deal with such issues by setting up committee of inquiry, in cases where a serious violation of scientific responsibility is suspected; by publicising the results of the inquiry in professional journals, and, if necessary, in the more popular journals and in the news media; and by calling the matter to the attention of governmental bodies", Edsall suggests, and he adds that "they can on occasion launch law suits on behalf of members who have apparently suffered injustice when acting on behalf of the public interest".

Although such activities may raise serious problems of funding and thus would require individuals to spend substantial amounts of time serving on hearing panels, Edsall reckons that "increased activity of the professional societies [is] the most hopeful approach to the problem in the immediate future". □

A SET of figures, published last week by the Department of the Interior, casts further doubts on the ability of the United States to shake off its dependence on imported oil. The department has drastically reduced its estimate of the amount of recoverable oil and gas that remains to be discovered in the United States and the new figures suggest that domestic resources could be severely depleted within about 30 years.

The new estimates are close to those published earlier this year by a committee of the National Academy of Sciences, on the basis of which the committee concluded that "US independence from external sources [of oil and gas] is essentially impossible on the basis of increased production of petroleum during the next decade".

The Department of the Interior now estimates that there are between 50×10^9 and 127×10^9 barrels of oil and between 320×10^{12} and 655×10^{12} cubic feet of natural gas yet to be discovered within the United States and, in deposits offshore. In addition, it reckons that about 30×10^9 barrels are recoverable from unexplored parts of existing fields.

Those figures stand in sharp contrast to estimates published a year ago which suggested that undiscovered oil reserves amount to between 200×10^9 and 400×10^9 barrels; gas reserves were put at between 990×10^{12} and $2,000 \times 10^{12}$ cubic feet. Moreover, in 1972, the Department of the Interior was predicting that undiscovered recoverable oil reserves amounted to 458×10^9 barrels. Those earlier estimates have, however, come under sharp attack from the oil industry because they were based on allegedly unrealistic estimating techniques, and the new estimates are derived by a method which the oil companies—and the committee of the National Academy of Sciences—believe is more accurate.

If the new estimates turn out to be correct, they hold a number of very important implications for energy policy in the United States. First, they dramatically underline the need for increased conservation of oil and gas. And, second, they make more urgent the need to develop replacements for natural gas and petroleum, such as synthetic fuels produced from coal. As Dr Vincent E. McKelvey, Director of the US Geological Survey, which developed the estimates for the Department of the Interior, put it last week: "[they] show that it is necessary soon to develop other sources of energy as the mainstay of our future energy supply".

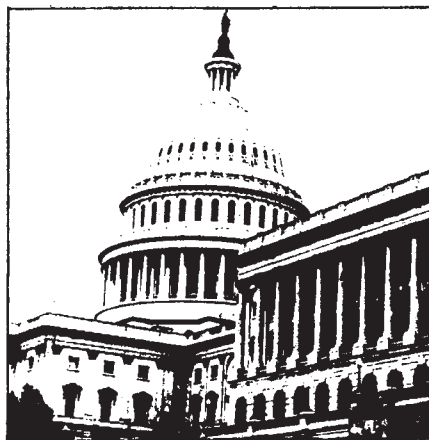
● In a move hailed as a major victory by environmentalist organisations and as a major tragedy by nuclear power

industry, the Nuclear Regulatory Commission (NRC)—which recently assumed the regulatory functions of the defunct Atomic Energy Commission—has announced that it will postpone, for up to three years, a final decision on whether plutonium will be allowed as a fuel for nuclear power plants in the United States.

Plutonium, which is a by-product of the fission reaction in nuclear power plants, could be recycled as a nuclear fuel to help eke out domestic supplies of uranium in the United States. The former Atomic Energy Commission had hoped to make a final ruling this year

Washington seen

from Colin Norman



on whether or not plutonium recycling should be allowed, but the NRC has now decided that the whole matter should be the subject of public hearings.

According to critics and supporters of nuclear power alike, the NRC's final decision on plutonium recycling will be crucial to the development of the nuclear power programme. For one thing, plutonium recycling would more than double the amount of energy which could be extracted from a given amount of uranium and it would therefore help hold down the costs of electricity production. And, for another, part of the justification for the breeder reactor rests on the fact that he plutonium produced in breeders would be used to fuel light water reactors. As Thomas Cochran, who works for the Natural Resources Defense Council, pointed out last week, "the breeder programme would look a bit silly if plutonium were banned as a fuel in light water reactors".

The chief objections to plutonium recycling are that it would involve transporting large amounts of plutonium around the United States. Since plutonium is highly toxic, and since it could be used as the core of a nuclear

explosive, opponents have argued that plutonium recycling would increase health hazards and greatly elevate the risks that a terrorist group could make off with a few critical masses of potential bomb material.

Environmentalists are pleased with the NRC's decision to hold public hearings before making a final ruling, because they are convinced that the delay will allow opposition to increase. The nuclear power industry, on the other hand, is less enthusiastic. Mr Carl Walske, President of the Atomic Industrial Forum, last week called the decision "ironic and deplorable", particularly in view of the Interior Department's recent drastic reduction in its estimates of oil and gas reserves in the United States.

The decision to postpone a final ruling on plutonium recycling may, however, turn out to be a shrewd political move on the NRC's part. It was the first major decision taken by the commission, and by opening up the issue to further public debate the NRC has served notice that it is prepared to take a tough stand in spite of vocal opposition from the nuclear industry. Its predecessor, the Atomic Energy Commission, was constantly accused of promoting the nuclear industry instead of regulating it.

● The National Aeronautics and Space Administration is moving into the final stages of planning a space mission to Uranus in the 1980s. Last week, NASA invited scientists to propose experiments for the mission, which would probably be launched in 1979, swing past Jupiter in 1981 and reach Uranus in 1985.

As now planned, the mission would use a Mariner-type spacecraft, which would pass Jupiter at a distance of about 392,000 kilometres and come as close as 24,000 km to Uranus.

Because Uranus has many interesting and enigmatic features—such as the fact that it spins on its side and its atmosphere seems to contain large amounts of methane—the mission has strong support among space scientists. But, earlier this year, the National Academy of Sciences suggested that NASA should reconsider the mission's priority in light of the fact that the space science budget is unlikely to grow much, if at all, in the coming year. Officials of NASA believe, however, that the mission could be fitted into the budget for two reasons. First, it will rely partly on existing hardware, which will keep the costs down and, second, the major expenses will be incurred before the most expensive development phase of other space missions such as the Large Space Telescope and a possible Jupiter orbiter mission.

Porton lab will study fever virus

by Eleanor Lawrence

THE new laboratory at the Microbiological Research Establishment, Porton Down, formally opened last week by Lord Godber, provides facilities unrivalled in Western Europe for the safe handling of the most dangerous viruses known.

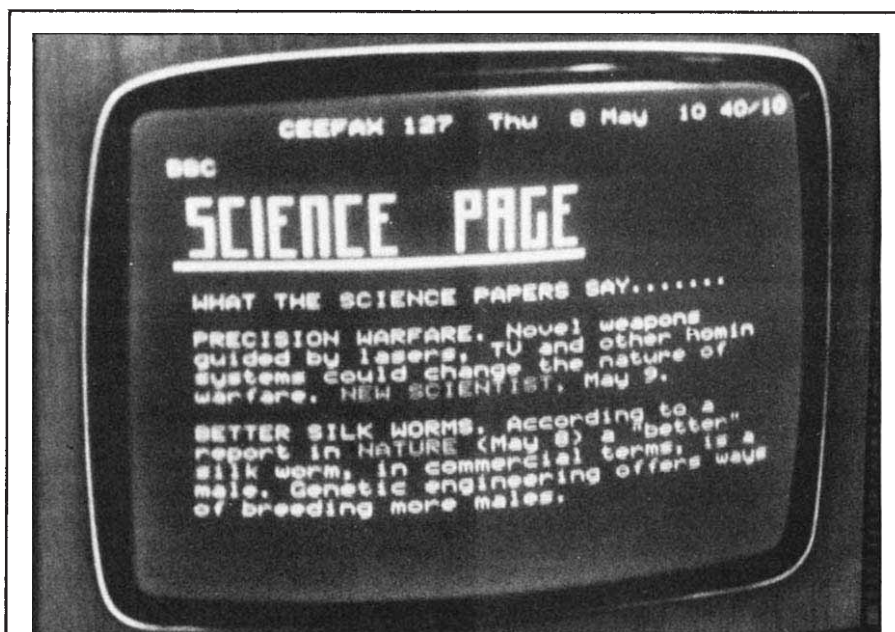
The new facilities will be used first to study the Lassa fever virus, and the other haemorrhagic fever viruses which have mainly been discovered within the past ten years and are virtually untreatable.

Porton, with a long history of handling some of the most dangerous organisms known to man was the obvious location for such a centre. The new laboratory was started two years ago, and has been modelled on facilities at the Communicable Diseases Center at Atlanta, Georgia, which, until now, was the only laboratory considered sufficiently secure to handle the Lassa fever virus.

The first task of the laboratory will be to build up a bank of diagnostic sera against the rare haemorrhagic fevers, such as Bolivian and Congo haemorrhagic fevers, and other exotic tropical virus diseases, such as Marburg disease, that may be brought into the UK. It will also provide this facility for other European countries and, in conjunction with Atlanta, for the areas where these diseases are endemic.

Lassa fever (first recognised in Nigeria) seems to be one of the most virulent virus diseases yet discovered. The mortality in Europeans with diagnosed Lassa fever is around 48% (although it seems to be lower in the native Nigerian population). It is also one of the most infectious virus diseases known. Patients shed virus from all body secretions and through the respiratory tract, and specimens of blood and urine are heavily infected. When the virus is multiplied up in tissue culture or in experimental animals, the risk of infection is greatly magnified.

Apart from its primary use in dealing with the virulent tropical viruses which may be carried into the UK undetected at any time by people returning from abroad, the laboratory will undoubtedly be in demand from research workers wishing to carry out some of the more potentially dangerous genetic engineering experiments in which the DNA of bacteria or viruses is modified by the addition of foreign DNA from other bacteria, viruses or even higher animals. Professor Robert Harris, the Director of Porton, said last week that he had been approached by the Ashby Committee and had argued that the laboratory facilities could be placed at the



THE BBC has been providing at first 24 and now 50 televised "pages" of printed material by using spare lines in the ordinary BBC 1 TV channel since last September; the service (code-named CEEFAX) is soon to be increased to 100 pages, and a similar service (called Oracle, but using the same standard technology as CEEFAX) will be provided by the Independent Broadcasting Authority (IBA) from next month. Until recently, the cost of a decoder to enable these printed words to be read on an ordinary television set has been prohibitive—at least £300 for a custom-made box of electronic tricks. But last week Texas Instruments, Bedford, announced in London that

they will be producing appropriate decoders on a production-line basis by early next year, when they should be available for about £50. A television set with such a decoder built in at the construction stage should then cost about £100 more than an equivalent set without a decoder, and the industry hopes that prices will fall.

As well as newflashes, sports results, a science page (see above), weather maps and other services already available from CEEFAX, this new technology opens up intriguing possibilities. Using telephone links and a keyboard, this kind of 'teletext' could help to create a home computer terminal, and there are obvious potential applications in education.

disposal of research workers wishing to undertake such experiments, but that he had not yet received any specific proposals. As the laboratory is sufficiently secure to satisfy the most stringent criteria set down by the recent conference called by Professor Paul Berg in California early this year, proposals will probably not be long in materialising.

The Berg conference recommended that experiments in which hybrids of two different viruses were made, or virus DNA (or DNA transcribed from RNA viruses) was linked to bacterial plasmids to multiply up virus genes, or mammalian DNA was linked to bacterial DNA could be carried out under conditions slightly less stringent than those in the new laboratory at Porton but that where the hybrid or the starting organisms were virulent or likely to prove so, the maximum security precautions should be taken. The other proviso of the Berg recommendations was that genetically enfeebled strains should be used.

But the prime object of the new facilities is the identification of the rare but virulent tropical viruses, which pose not just a potential but an actual public health hazard. With aeroplanes bringing their human and animal cargo into European airports every day from the tropics, the possibility of an epidemic of one of these virulent untreatable viruses cannot be discounted.

The only specific therapy which has proved effective against Lassa fever is serum taken from a convalescent patient. But with only three Europeans having recovered from the disease, the supply of convalescent serum is extremely limited. Also, administering convalescent serum from a newly recovered patient can be extremely risky as virus is excreted from the patient for several weeks after clinical recovery. One aim at Porton is to establish a bank of safe serum, either from convalescent patients or from the naturally resistant native population, which can be given to patients once the disease has been diagnosed. □

Trying to get MPs interested in science

from David Spurgeon, Ottawa

FIVE years ago, when interest in science policy was still running high among some Canadian scientists and politicians, the Association of the Scientific, Engineering and Technological Community of Canada (mercifully shortened in popular parlance to SCITEC) was formed. It was established in response to the frustrated complaint of a member of the Senate Special Committee on Science Policy that he could not determine which among the many voices really spoke for science in Canada. It was to become a sort of parliament of science, and one of its main purposes was to initiate a dialogue with members of the real Parliament in Ottawa.

Since those palmy days, science policy discussions have fallen upon such bad times that when SCITEC recently held its fifth annual meeting, one parliamentarian summed up the present situation thus:

"Science policy is the biggest non-question in the House of Commons. Nobody even knows how to frame a question. On occasions I have been asked to please ask a question of the science minister. Nobody even knows he's there, and a politician can stand anything except not being noticed."

Max Saltsman, New Democratic Party member, had been invited as a member of a panel held after the annual meeting to initiate between parliamentarians and scientists, just the kind of dialogue both have found lacking in the past. The report of the outgoing association president, Dr M. P. Bachynski, said it was SCITEC's intention "that this become a continuing dialogue which could lead to a more formalised vehicle for such interaction—an association of scientists, engineers and parliamentarians."

This hope reflected an earlier one, expressed years ago when the organisation had just got going, that an in-

formal club might be formed for the same purpose. Under the circumstances, the hope seemed rather a forlorn one.

But at least a dialogue had begun. Most of the MPs on the panel, of course, might have been expected to be interested, because they had scientific or engineering backgrounds. They included Harvie Andre, a Progressive Conservative MP with a PhD degree in chemical engineering, formerly an associate professor in that discipline; Frank W. Maine, Liberal MP and former Athlone Fellow, who holds a PhD in organic chemistry and was previously director of research and development in a manufacturing company; and Ross Milne, Liberal, an engineer with a master's degree in environmental control.

Mr Andre was particularly concerned with the present state of public policy relating to science: he called it "a hell of a mess." Recalling the great interest in science policy during the 1960s, he said this had led to an uncomplimentary comparison of Canada's performance with that of other industrialised countries. A whole series of studies had shown that Canada spent proportionately less on research and development than most industrialised nations, and spent less on such activities in industry and more on in-house government research.

The response of the federal government was to create the Ministry of State for Science and Technology (MOSST), but that did not solve the problems, Mr Andre said. "In the four years that department has been in operation, in every way, in each of those [four] areas of comparison, we are worse off." Canada now spends a smaller percentage of its GNP on science and technology; in spite of the MOSST's contracting-out policy, the proportion of in-house research and development has increased rather than decreased (from 65 to 72% of the total); and the percentage of the GNP spent on research and development by industry has about halved.

Mr Andre said he was not implying this was all the MOSST's doing. He felt

rather that the ministry lacked muscle to accomplish its objectives. He would like to see it obtain that power—if not, it should be abolished. "It is now a convenient screen to divert attention away from problems and it allows the government to say they are curing the problems."

Dr Virginia Douglas, immediate past president of SCITEC and a panel member representing science, said politicians and scientists both seem to agree that they must get together, yet they also seem to agree that, after endless studies, "we've achieved little communication."

She suggested the trouble was that the two groups work from different assumptions and use different techniques to solve problems. She said the mission-oriented approach is understandably attractive to politicians, but many others are not convinced that a direct, simple-minded approach will solve society's problems. Scientists are being asked to provide simple answers for complex questions—sometimes insoluble ones. And not everything can be subjected usefully to cost-benefit analysis, as politicians would like.

The attempt to control science and put it to work through bureaucracy does not seem to be working, she said, and it endangers the productivity of science. She pleaded with the politicians not to put scientists into a bureaucratic strait-jacket and not to put them into the position of being adversaries. And she said the time has come to stop talking abstractly about policies and start adopting a case approach to the solution problems.

Perhaps one of the best indications of the parlous state of relations between SCITEC and the federal government was revealed in Dr Bachynski's report on SCITEC's attempt to obtain approval of a plan to build a house of science and technology (HOST) to bring together the mostly indigent scientific societies centrally in Ottawa.

The MOSST had never answered the proposal officially, Dr Bachynski said. But unofficially, the indications were that the ministry would not support it as SCITEC wanted. "The impression I have is that the ministry would be glad to support such a project as long as it didn't cost the government any money."

Money, of course, was exactly what SCITEC and the other societies needed. But, according to Dr Bachynski, the ministry seemed to think that it would be a difficult idea to sell politically, because all kinds of other groups might then want similar favours and the electorate would not see why scientists should be treated any differently from anyone else. A perfectly understandable view, one is tempted to add. □

Clearing house for cancer epidemiology

THE International Agency for Research on Cancer of the World Health Organisation in Lyon is collaborating with the Deutsches Krebsforschungszentrum in Heidelberg in setting up an international clearing house for the exchange of information on epidemiological studies in cancer. Requests are just going out to epidemiologists to complete a standard form describing the

on-going projects. It is hoped that in this way an Annual Directory can be compiled and also that participants can be supplied with current information at any time. The sponsors believe that this may, among other things, help cut down the number of occasions on which similar studies cannot be compared because some relatively minor procedure was not carried out in all cases.

THE publication in the United States last week of tapes purporting to reveal the "last hours" of Colonel Vladimir Komarov, who perished aboard Soyuz 1, can hardly be considered an auspicious augury for the forthcoming Soyuz-Apollo programme. The tapes raise the whole problem of credibility and completeness of information exchange between the Soviet and American participants.

According to the tapes, the Soyuz was out of control for some 12 hours before the disaster, with Komarov fighting desperately to retain control (taking time off, however, for a farewell to his wife and child, and a talk with Premier Kosygin). According to the official Soviet releases, the flight proceeded normally until the moment of deployment of the parachutes. The Tass communique (April 25, 1967) stated: "After implementation of all the operations connected with transition to the landing regime, the ship successfully completed the most difficult and responsible braking sector in the dense strata of the atmosphere, and fully decelerated from the initial orbital velocity. However, when the main parachute was opened at an altitude of 7 km, a twisting of the parachute lines, according to preliminary data, caused the ship to descend at a great speed which resulted in V. I. Komarov's death".

An article by Yuri Gagarin, who was present at the control centre during the whole re-entry procedure, said much the same. The flight was perfectly normal, he maintained, until the moment when the parachutes should have opened. He described the shock felt by all in the control centre when this last-minute disaster occurred. Gagarin's article appeared in *Komsomolskaya Pravda*, the newspaper of the Young Communist movement (young, in this context, meaning 18-35 years).

Certainly, a number of Western commentators at the time suggested that the spacecraft had been out of control for some time before re-entry—these commentators, however, seemed motivated by a preconceived idea that, since "Soyuz" means union, Komarov's craft was meant to be a participant in a manned link-up, but that difficulties with his craft led to the cancellation of the (presumed) second launch and his "premature" re-entry after only 18 orbits.

Another mystery is the state of Komarov's health. A background article in *Pravda* of April 24, 1967, which had gone to press before the disaster, stated that after the flight of Vostok 4 (for which he was back-up for Popovich), he had developed a heart murmur, but "a good rest, a

more sensible everyday regime, self-confidence and the moral support of his comrades did their work", so that a later medical board found nothing wrong with him, and he was able to make a 24-hour orbital flight in October 1964. Before the flight of Soyuz 1, said this article, he had received "the highest rating". Certainly, it would seem unlikely that any space programme would launch a man in less than perfect health—yet some medical reports which seem to have been written for limited circulation among Soviet experts in space medicine seemed to imply that the proximate cause of the disaster was that Komarov

Soviet diary

from Vera Rich

lost consciousness at a crucial moment during re-entry, implying either that the parachute system had to be deployed by a manual control (out of key with the whole tenor of the Soviet space programme, which places so much emphasis on automation that the early cosmonauts were virtually no more than human test animals) or else that, being unconscious when disaster struck, he was unable to make some last minute effort to save himself.

● One of the contributions of the Byelorussian SSR in the current five-year plan is an extensive programme of the study and conservation of the flora and fauna of the republic. This included the launching of a new popular science journal *Rodnaya Pryroda* (Our native nature), research projects by the various scientific institutes of the republic, and a special prize for the best coverage in the media of the problems of conservation. The programme was clearly aimed at local loyalties and sentiment, with extensive use of the Byelorussian language (although current Soviet policy favours the "unifying" importance of Russian).

According to reports in *Rodnaya Pryroda*, the programme is making excellent progress, no less than 13 of the 48 scheduled "themes" being completed three years ahead of schedule. In at least one respect, however, all is not well with conservation in Byelorussia—the close season for fishing is not being respected. During the spawning season, persons unspecified are "barbarously" fishing for shallow water fish, to the great detriment of the fish reserves of the republic.

To combat this, "all possible forces" are to be mobilised, ranging from the Fisheries Board of the Council of Ministers of the Byelorussian SSR, through the Byelorussian Conservation Society and the Society of Hunters and

Fisherman, down to school-children and the "pioneers" youth organisation. How, precisely, they are to combat the poachers is not explained—presumably by some attempt at patrolling the numerous lakes and rivers of the aquiferous republic. What is more puzzling is who is doing the poaching at all. Byelorussia is not rich in natural resources, and has a long peasant tradition of cautious preservation of the little that is available. Although the new great oil refineries at Novo-Polotsk and Mozyr have brought in many workers from all parts of the Soviet Union, it would be too naive a solution to attribute the depredations to oilmen having a quiet day's angling. The most probable answer seems to be that of 'deculturalisation'—that state planning and state control have destroyed the old, careful traditions, and that rules of conservation which were once an integral part of local tradition now have to be reimposed, by official decree.

● The idea of recovering gold from the sea is not a new one. But attempts to date have all proved uneconomical—the value of the metal recovered being less than the cost of extraction. Now, however, data from the recent Atlantic voyage of the research vessel Mikhail Lomonosov have reopened the question of extracting gold from seawater. Analysis of deep-water samples indicate a gold concentration some 1,000 times higher than expected—a value which, if confirmed for a sufficient number of sites, might well indicate that the metal could be extracted commercially.

The reasons for these high concentrations are as yet a mystery and, not surprisingly, the sites of these anomalies have not been revealed.

● A new means of producing extra-strong concrete has been developed at Naovi (Uzbek SSR)—dropping it. This, according to the inventor, Candidate of Technical Sciences A. K. Brovtsyn, produces "complex physico-technical interactions between the grains of the filler and the cement particles", giving an increase in strength of up to 15%. A pilot plant for dropping concrete components of up to 3 tonnes in weight on to an elastic base from a height of 35 cm has been developed at a cost of 2,000 roubles (£1,000).

● An artificial method of producing triplets from single-yoked eggs has been demonstrated by embryologist, German Syatogor of Leningrad University. The method consists of surgically opening the egg, dividing the blastocyst with a micro-scalpel, and then resealing the egg. So far, the Novosti agency reports, triplets have been successfully produced, from the eggs of hens, ducks, turkeys and Japanese quail.

correspondence

LEPRA'S money

SIR,—In writing on leprosy (March 20), Dr Crawford claims that the numbers on treatment have remained low for the reasons he mentions and not for lack of money, as LEPRA claims.

The cost of maintaining patients in institutions is enormously high and LEPRA recognised this in 1963 when it carried out a reappraisal of its work. Consequently it has not for many years given grants for maintaining patients unnecessarily in institutions, but still maintains that the numbers on treatment have remained low because of a lack of money, as the cost of finding patients and ensuring their regular treatment on a domiciliary basis for at least three years is very high.

Dr Crawford concludes his article with an accurate example of how LEPRA is using a very small proportion of the money given to it, for a specific purpose. His example, however, may have given your readers an inaccurate picture since the example suggests that voluntary agencies in Europe are still spending most of their income on institutional care. This is not the case with LEPRA since its contribution to the fight against leprosy in 1974 was:

- £210,000 on leprosy control projects, mainly for domiciliary treatment.
- £66,000 on grants to encourage early diagnosis for domiciliary treatment of children suffering from leprosy.
- £10,500 on leprosy research.
- £6,600 on training.
- £6,500 on the leprosy aspect of multi-purpose rural health projects.

G. F. HARRIS

LEPRA, Colchester, UK

HTRs, zink and aluminium

SIR,—The high temperature gas-cooled reactor (HTR) has frequently been suggested as a source of process heat for steel-making, but we suggest that it could also be used in the zinc—and aluminium—making processes. The key step is the reduction of zinc oxide using a gas heated by an HTR.

At present there are two principal techniques involved in the zinc-making process: chemical reduction using coal, and electrolysis of zinc oxide. The first is carried out at temperatures of 1,200–1,400 °C and needs a correspondingly large supply of coke to provide these high temperatures. Zinc oxide might, however, conveniently be reduced using methane or hydrogen with an HTR as

the source of process heat. In this case temperatures much below 1,000 °C are necessary—and these can be delivered by today's HTR technology. Natural gas or methane produced by coal gasification using HTR process heat can then be heated by the HTR to 800 °C and can be used for the reduction of zinc oxide. If this reduction has to be done with hydrogen, the latter might be produced by steam reforming of methane or water splitting, using process heat.

The zinc metal produced can either be sold or used in the aluminium-making process. Aluminium is at present produced by molten-salt electrolysis of alumina but high electrical energy requirements and high investment costs are disadvantages of this method. A new purely chemical reduction method, the so-called Toth process, seems to avoid these drawbacks. It is based on a four-step process in which aluminium metal is obtained from an ore containing alumina by way of aluminium chloride. Aluminium chloride is reduced with manganese metal yielding an aluminium–manganese alloy and manganese chloride, which is subsequently oxidised with air and finally reduced with coke at 1,750 °C.

Zinc might be used instead as reducing metal. In contrast to manganese oxide, the zinc oxide formed can be reduced by hydrogen or methane at temperatures far below 1,000 °C.

S. HUWYLER & W. SEIFRITZ

Würenlingen, Switzerland

Nitrogen fixation

SIR,—Does A. B. Lovins (May 1) really believe that those working on nitrogen fixation have not tried to imagine what would happen if they succeeded “beyond one's wildest dreams”?

What if one did develop universal nitrogen-fixing systems for plant life on this planet? The effect would be the same as if adequate conventional nitrogenous fertiliser were universally available: light, water, nutrients other than N (K, P, S, Co, Mo and so on) would become limiting, depending on the particular environments. Assuming all these deficiencies could be corrected artificially, CO₂ fixation would be the final limiting factor—as it is in advanced agricultural industry already.

The ecological and environmental effects would be just about the same as the use of nitrogenous fertiliser has entrained already, but the drain on

the world's energy resources (for fertiliser production) would certainly be very much less. The pollution problem arising from run-off might also be lessened, though not eliminated—rotting legumes produce a run-off problem even today. In compensation, a few hundred million people on this planet would be better nourished and a few hundred thousand would be saved from death by starvation each year. They would, of course, increase and multiply, so a global problem would be augmented and its solution postponed rather than solved. Yet some feel that there are better ways of tackling the population problem than by accepting starvation.

JOHN POSTGATE

University of Sussex, UK

Ohta and evolution

SIR,—The paper by Ohta (November 29) on which my comments (April 3) have so displeased Nigel Calder (May 1), appeared under the rubric of “Review Article”, which might lead the uninitiated (including perhaps Mr Calder) to expect a more or less scholarly summary of recent evidence and theories. What Ohta gave us, in fact, was a highly partisan presentation of the case for one side only on a controversial issue.

My remarks on statistics were perhaps open to misunderstanding; it was not intended to imply that statistical methods have no place in evolutionary studies—for example the statistical work of R. A. Fisher was a very important contribution to the theory of natural selection—but merely to point out that, in the macroscopic world at least, the appeal to chance is essentially an argument from ignorance, and should not be made until causal explanations have been clearly shown to fail.

Mr Calder's reference to my remarks on scientific truths makes it clear that there are philosophical differences between us which would need more space for their discussion than this letter could afford. To quote a writer for whom Nigel Calder might have more respect than for the scriptures, William Blake wrote that “establishment of truth depends on destruction of falsehood continually”—in that spirit I was moved to express my criticism rather more forcefully than is usual in these columns.

R. A. CROWSON

University of Glasgow, UK

news and views

Sunspots and the solar cycle

from T. G. Cowling

It is generally agreed that sunspots are surface phenomena, and that their darkness is due primarily to their possessing strong magnetic fields. It is also agreed that bipolar sunspot pairs arise when a bundle of the field lines of a subsurface azimuthal magnetic field has broken through the Sun's visible surface to form a magnetic arch above the surface. Nearly every other assertion made about sunspots has recently been challenged.

In recent years the origin of solar magnetic fields has customarily been explained in terms of dynamo theory. This theory, originating as a modification of a similar theory for the Earth, begins by assuming a poloidal field (one in meridian planes) which is twisted by non-uniform rotation to yield a much stronger toroidal (azimuthal) field. The dynamo has to be completed by a feedback mechanism converting toroidal field back into poloidal; the reconversion cannot be effected by axisymmetrical motions. Parker¹ in 1955 was the first to suggest a suitable mechanism. He showed that the reconversion could be effected by twisting (by Coriolis forces) the field embedded in material being carried upward because of thermal convection in the Sun's outer layers.

Parker's original discussion rested on general physical principles rather than on detailed mathematical analysis. But any defect in this respect has been amply rectified by later workers like Braganskii², Steenbeck and Krause³, and Moffatt⁴, who have also suggested other ways of securing feedback. In particular, the mean-field electrodynamics of Steenbeck and Krause, with their isolation of the so-called α -effect (currents parallel to the mean magnetic field) has supplied both a justification for Parker's ideas and a genuinely useful mathematical structure for use in dynamo theory. This structure has been used with conspicuous success in mathematical work on the Earth's dynamo.

In certain respects solar dynamo theory has been similarly successful. For example, as Parker originally surmised, it gives a field progressing towards the Sun's equator, and reversing periodically; this agrees with the

observed progression of the sunspot belt towards the equator in any 11-year cycle, and the reversal of polarities in a bipolar spot pair in the next cycle. There is also some observational evidence of a reversing dipole field, at least in high latitudes. This field is, however, weak and uncertain; patches of both polarities are regularly present in each polar cap, and only in an average sense can one speak of the polarity of the cap as a whole. Surface observations provide little evidence of the regular dipole field required to initiate the dynamo cycle.

The observed field is represented more closely by a model suggested by Leighton⁵ in 1964, and given mathematical form by him in 1969. This makes use of the tendency of the following spot of a pair to appear at a rather higher latitude than the leader spot, together with the spreading of fields over the Sun's surface by a random walk; this mechanism is employed as a substitute for Parker's feedback process depending on Coriolis forces. Leighton was able to get a 'butterfly' diagram in good agreement with observation for the progression of the sunspot zones towards the equator in any cycle; dynamo theory based on the α -effect makes new spots of a cycle appear at excessively high latitudes. Leighton also found a reversal of the polar (dipole) fields two or three years after the beginning of a new cycle, as observed, whereas α -effect dynamos tend to make the two nearly simultaneous.

Part of Leighton's success was due to his introduction of 'threshold' terms effectively limiting field generation to low latitudes, and ensuring that high-latitude fields arise only through the random walk. Apart from these threshold effects, Leighton's equations closely resemble those of an α -effect dynamo and, as Stix⁶ recently showed, the α -effect dynamos can mimic Leighton's results if the law of non-uniform rotation is chosen such as to confine field generation to low latitudes. Leighton's discussion is in any case subject to the criticism that it relies over-heavily on purely surface observations, and gives little insight into the physics of the dynamo process

at greater depths. Since the latitude effect invoked by Leighton is itself likely to arise from Coriolis forces, it is reasonable in discussing physical processes to confine attention to α -effect dynamos resembling that of Parker.

Piddington⁷ has criticised such dynamos, on two main grounds. The first is that they create their new poloidal flux always at a higher level than the original flux, so that ultimately they should push the magnetic field out of the Sun. The second is that in order to get field reversal one needs not only to create new flux but to destroy unwanted old flux, and the electrical conductivity of solar material is too great to permit destruction in the relatively short times allowed. The reply of the dynamo theorists^{8,6} has been that ohmic dissipation is supplemented by turbulent diffusion, which brings oppositely directed fields into close juxtaposition, so facilitating their mutual annihilation. Turbulent diffusion is also regarded as able to bring new magnetic flux down to the level of the old.

Piddington refuses to accept this reply as valid. He points out that surface observations do not support the concept of violently tangled fields, but show large patches of one polarity existing side by side with similar patches of the opposite polarity; small-scale dissipation, such as Stix⁶ suggests on the basis of solar 'filigree' observations, can destroy small irregularities within the polarity patches but not destroy the patches themselves. He also argues that the concept of turbulent diffusion, though useful in discussing the transport of scalar quantities, is less satisfactory when discussing magnetic problems, where field lines have to be transported across field lines not in the same direction. Parker⁸ has discussed mechanisms for speeding up the relinking of field lines in such transport. It is uncertain whether these mechanisms can work below the Sun's surface; Piddington asserts that in any case they cannot work fast enough until the field has grown so strong as to be able to choke the convective motions supposed to generate it.

Thus, though the dynamo equations certainly do give solutions that agree with the observed fields, Piddington

asserts that these equations do not represent physical reality. Instead of the relatively diffuse fields of dynamo theory, he regards solar fields as composed of twisted 'ropes' (bundles of field lines) whose fields are strong enough to defy permanent distortion, and which are nearly indestructible. He quotes observational evidence in favour of this point of view. Away from sunspots, most of the surface magnetic flux is concentrated in small elements, with fields approaching 1,000G, situated at the junctions of supergranular convection cells. On dynamo theory such fields are supposed to be concentrated by the convection; he believes that they control it. More convincing evidence is provided by the facts that, when magnetic fields burst through the Sun's surface, they appear to do so as sets of individual ropes⁹; when a spot decays, it does so by peeling off ropes which are seen as magnetic 'knots' moving across a 'moat' surrounding the spot.¹⁰

Piddington envisages a sunspot magnetic field as a stranded cable of magnetic ropes. He regards twists of the ropes as essential, to avoid an ex-

change instability that would otherwise explode the field (however, this instability is not yet definitely established). He is unable to get a reversal of the Sun's field as a whole, and to this extent his ideas compare unsatisfactorily with those of dynamo theory. The best he can do is to create a reversing toroidal field from a non-reversing poloidal field limited to the deep interior, by a torsional oscillation similar to that originally considered by Walén¹¹ in 1948. As Stix⁶ has emphasised, this idea meets with a number of difficulties, not the least being that it postulates a subsurface field unrelated to anything that we actually observe.

But the dynamo theory is subject to a similar objection. The polar field observed on the Sun is not the regular poloidal field posited by dynamo theory as its starting point; it seems rather to be an epiphenomenon, left over from sunspot fields. Also, as Altschuler *et al.* have shown¹², and as is confirmed by observations of the sector structure of interplanetary fields¹³, the dominant large-scale field of the Sun rarely is that of an axial dipole;

more often it is that of an equatorial dipole or quadrupole. Thus, though the remarkable success of dynamo theory as a mathematical theory must be acknowledged, doubts must persist whether it explains the actual solar fields. One cannot but wish that one could look beyond the Sun's outermost atmosphere, and see what is going on at greater depths.

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Drugs and nucleic acids

from Stephen Neidle

THE nucleic acids DNA and RNA are undoubtedly among the best defined of all target molecules for the action of drugs, antibiotics and anti-bacterial agents. This has encouraged very many attempts over the past twenty years to define the molecular geometries of such drug-nucleic acid interactions, often with the ultimate hope of more effective and rational drug design.

It is nearly fifteen years since Lerman proposed his intercalation model for the interaction of aminoacridine mutagens, such as proflavine, with DNA; this model has survived the test of time rather well, at least in general terms. Lerman proposed that the planar drug molecule could slot (that is, intercalate) between the base pairs of DNA, with consequent unwinding of the double helix. He and numerous other workers have demonstrated by a variety of physical methods that many of the changes in DNA properties observed on binding can be directly attributed to intercalation. Unfortunately, although it has been established that helical unwinding does occur, the precise degree of unwinding has long been a point of uncertainty. Estimates have varied from 12° to 45° per drug insertion. Only very recently has the matter been clarified; the precise studies of Wang, using a buoyant density gradient caesium chloride titration method, have

indicated that the correct value is 26° (*J. molec. Biol.*, **89**, 783; 1974). Although Wang's studies were on the DNA-ethidium system, there is good reason to believe that many other intercalating drugs untwist the helix by a similar amount.

Support for the intercalation model has now come from the crystallographic analyses of Sobell and his co-workers. They have capitalised on several recent findings that self-complementary dinucleoside monophosphates, such as adenylyl-(3', 5')-uridine, form Watson-Crick hydrogen-bonded dimers in the solid state, which can be considered to be RNA double-helical fragments. Sobell *et al.* have therefore used such fragments as nucleic acid models, and they have complexed them with the trypanosidal drug ethidium bromide, a well established intercalating agent. The preparation of complexes with 5-iodouridylyl-(3', 5')-adenosine and cytidylyl-(3', 5')-guanosine was briefly reported at a recent Royal Society Discussion Meeting, and Tsai, Jain and Sobell have now published an account of their X-ray structural studies on the former (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 628; 1975). The structural unit consists of a Watson-Crick base-paired dimer, to which is attached two ethidium ions. One of these is positioned between the

base pairs of the now opened-up miniature double helix, and the other stacks externally on top of them. The intercalated ethidium has unwound the dinucleoside phosphate by about 12° compared with its uncomplexed conformation—this corresponds to unwinding angles of 26° for RNA, and 29° for DNA, assuming a direct correlation between model and polymer (nucleic acid) intercalation. As the authors do indeed point out, this assumption is unproven, in spite of the satisfying agreement of their result with Wang's.

There are in fact several features of this intercalative complex structure that may well belong merely to very small nucleic acid fragments, as well as to this particular analysis. Such a feature is the unexpected mixture of nucleotide sugar conformations, which could well have a significant effect on the unwinding angle. This structure forms an interesting contrast to that recently reported for another dinucleoside monophosphate-drug complex (Seeman, Day and Rich, *Nature*, **253**, 324; 1975), between the mutagen 9-aminoacridine and adenylyl-(3', 5')-uridine; this complex has an open, non-double-helical structure with drug molecules stacking on either side of, this time, Hoogsteen-type adenine-uracil hydrogen bonds in contrast to Watson-Crick base-paired

adenylyl-(3', 5')-uridine itself. It is likely that the complex interplay of intra-molecular forces which serves to stabilise double-helical nucleic acids is finely balanced for dinucleoside monophosphates. There seems to be no guarantee that these can preserve their 'mini nucleic acid' features when complexed with drugs. Probably only rather longer complementary oligonucleotides will satisfactorily reveal the molecular features of drug intercalation, especially subtle cooperativity and nearest-neighbour effects.

Prominent among many putative intercalating drugs is the plant alkaloid ellipticine, which has a planar ring structure capable of being inserted between base pairs. Ellipticine has received widespread attention on account of its high anti-leukaemia activity, which is believed to be related to its binding to nucleic acids (compare other anti-cancer drugs, such as actinomycin and daunomycin). Kohn, Waring and their co-workers have now established that this drug does indeed bind to DNA by an intercalative process (*Cancer Res.*, **35**, 71; 1975). The unwinding angle is less than that for ethidium, but very close to that for proflavine, another classical intercalating agent. Indeed ellipticine and proflavine have generally very similar binding behaviour, at least with calf thymus DNA, presumably because their molecular dimensions are so similar.

Le Pecq, Xuong, Gosse and Paoletti have used the intercalating properties of ellipticine in a systematic attempt to obtain highly active anti-cancer drugs by consideration of various ellipticine molecular modifications (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 5078; 1974). Their very simple reasoning was that, assuming DNA is the genuine cellular receptor for these drugs, a high degree of binding could correlate with high anti-tumour activity. DNA-binding constants and unwinding angles, and activity against L1210 mouse leukaemia, were determined for a series of derivatives; 9-hydroxyellipticine had the highest DNA affinity, and moreover had the strongest pharmacological activity. It seems plausible that the introduction of the hydroxy group has enhanced binding, by means of a hydrogen bond to a DNA phosphate group.

Of course, as Le Pecq *et al.* point out, strong binding to DNA does not by itself confer anti-tumour activity, as opposed to other biological activity—one of the derivatives tested by Le Pecq *et al.* had marked trypanocidal properties only. Many other molecular and indeed cellular properties are important. But one can certainly look forward to other successful applications of this straightforward approach to drug design.

A new theory of the Universe

from P. C. W. Davies

FRED HOYLE has long been known as a man with provocative and radical ideas about the nature of the Universe. During the 1950s and early 1960s he championed the famous steady-state theory of cosmology, in which the gross features of the Universe were assumed to remain unchanged for all eternity, a situation to be maintained by the continual creation of matter to fill the gaps left as the Universe expands.

This theory proved highly attractive philosophically, and was for a time very popular among cosmologists, but towards the end of the last decade, mounting observational evidence seemed to indicate that, rather than remaining unchanged, the Universe had a birth, growth and death, after the fashion of a biological organism. The 'big bang' theory is based on the assumption that the Universe was created about 15,000 million years ago in a hot, dense and violent expansion. A faint glow from this initial fireball is still detectable today, in the form of thermal radiation bathing the Universe at a feeble three degrees above absolute zero. This radiation is known in the trade as the microwave background.

Latterly, Professor Hoyle has accepted the concept of a hot big bang, but in a recent paper in *The Astrophysical Journal*, he describes a new and somewhat unusual perspective on this momentous event. In order to understand this departure, it is necessary to know something of the theoretical background to cosmology which Hoyle has developed with his co-worker Jayant Narlikar.

A perennial headache among physical scientists is to know which system of units to adopt for measurement of physical quantities. As it happens, nature supplies us with certain universal quantities against which it appears natural to measure everything else. Two such quantities are Planck's constant and the speed of light. Accepting these as our basic units of measurement, all other physical quantities may then be measured in terms of mass. For example, the mass of the electron is associated with a length of about 10^{-13} cm and a time interval of about 10^{-23} s.

Now it is generally accepted that the Universe is in a state of expansion, which is best visualised as a systematic and uniform growth in the scale of cosmic distances. To be specific, every day the observable Universe grows in scale by 10^{16} cm, or about 10^{29} electron mass units. This steady growth implies that in the past the Universe was shrunken, and simple versions of the

big bang theory demand that 15,000 million years ago the shrinkage was complete, with the whole of the Universe which we at present observe squeezed up into a single point. This awesome event is generally known as a singularity, and is usually identified with the creation of the Universe. Certainly physical reasoning cannot be extended back through such a singularity.

The novel proposal made by Hoyle is to turn the cosmic scale change around, and regard the mass as the quantity which varies while the Universe remains static. Such a situation can be procured (mathematically) by changing the scale of measurement at different moments of time, something known to mathematicians as a conformal transformation. By thus transforming away the cosmological motion "The usual mysteries concerning the so-called origin of the Universe begin now to dissolve", according to Hoyle.

Lest the reader be suspicious about the creation of the Universe being removed at a (merely mathematical) stroke, even from the pen of so auspicious an individual as Professor Hoyle, something of the theoretical framework surrounding this new perspective should be mentioned. The use of conformal transformations to remodel the geometry of the Universe is often used for convenience of mathematical discussion. In this way the violent shrinkage of space near the big bang can be



A hundred years ago

THE new Reptile House in the Jardin des Plantes, Paris, has sustained some heavy losses. A large turtle died from the shot it had received many months ago when captured in the Atlantic Ocean, and a large serpent from a wound inflicted by a rat. The rat having been offered as living food, resisted violently, and bit his adversary so deeply that he died a few days afterwards. The wardens in the picturesque Reptile House will probably be more cautious in future in showing visitors the spectacle of Ophidians running after their food.

We are glad to say, however, that the above heavy loss will be to a considerable extent compensated, as the Jardin des Plantes will receive in a very few days a Boa more than eight yards in length, which has just arrived at Havre. We believe it takes a goat or a sheep to appease its appetite at one time.

from *Nature*, **12**, 54; May 20, 1875

'stretched out' again. The physical relevance of these transformations is usually limited, though, to considerations of systems without mass (for example, electromagnetism), simply because a non-zero mass changes under the transformation, and this is something not normally encompassed by conventional theories. But Hoyle appeals to a theory of mass and gravitation which he developed some years ago with Narlikar. In this theory, the mass of a particle is not a fixed, intrinsic attribute, but arises by an interaction between the particle and a 'mass field' which is in turn produced by all the other matter in the Universe, in a fashion often associated with the ideas of Mach. As a result, the mass field (and hence the particle mass) may vary from place to place, and time to time. By thus making variable mass a foundation stone of the theory, the use of conformal transformations fits quite naturally into the scheme. Not only massless systems, but all of physics, now become invariant under these transformations.

The utility of this extra flexibility is that the physicist is free to choose which conformal frame to work in. For example, by choosing the masses to be constant, the usual picture of an expanding Universe is obtained, and indeed the Hoyle-Narlikar theory then reduces to the conventional general theory of relativity. The singularity which occurs at the beginning of the big bang in this picture cannot be avoided in general relativity as a real physical catastrophe. But in the Hoyle-Narlikar theory this event merely signals a mathematical breakdown in the fixed mass frame, which may be neatly side-stepped by conformally transforming to the fixed Universe-varying mass frame.

The 'creation of the Universe' is then relegated simply to a moment when all masses happen to vanish, something which occurs quite naturally from time to time, according to Hoyle. The reason for this is that there exist both positive and negative sources of the mass field (reminiscent of positive and negative charges in electrodynamics), so that now and again an exact cancellation may occur.

Having thus argued that the big bang was not a creation event at all, Hoyle then goes on to ask the question: what happened before? In a brief discussion, some of the features which we now observe in the Universe and attribute to unknown properties of the big bang, are connected instead with processes occurring in an earlier phase. Some of the suggestions are not new, such as the energy to heat the fireball (visible now as the microwave background) originating from the accumulated starlight of an earlier phase.

Others, such as the preservation of some residual stellar structure through the big bang/mass vanishing phase are quite unusual. The latter is used as an explanation of the deuterium to hydrogen ratio in the Universe, by speculating that synthesis of deuterium took place in regions of much lower density than average.

In summary, Hoyle has produced a picture of the cosmos which bears some similarity to the steady-state theory (no creation event) but still incorporates the essential features of the big bang fireball. The vital ingredient of the steady-state theory—the continual creation of matter to maintain constant entropy conditions—is not present, however. Consequently the new model suffers from the usual paradox of all infinite age cosmologies: why haven't irreversible processes generated unlimited entropy? Shouldn't the accumulated starlight in the fireball be arbitrarily hot from an infinity of previous phases? Nevertheless, many physicists may find this imaginative new picture a comforting circumvention of the unpleasantness of the initial singularity.

Disagreements over Troodos

from Peter J. Smith

DURING the past decade or so, almost all earth scientists with experience of the Troodos Massif of Cyprus have managed to convince themselves (and most others) that this ophiolite sequence is an upthrust fragment of ancient ocean floor and thus originally formed at an ocean ridge. It is a remarkable achievement, not least because the evidence, though strong and diverse, is almost entirely circumstantial; and the data required to remove all shadow of doubt remain to be found. In 1973, however, the consensus which had taken so long to build up was abruptly shattered by Miyashiro (*Earth planet. Sci. Lett.*, **19**, 218; 1973) who proposed instead that Troodos was created as a volcano in an island arc between Eurasia and Africa. The verbal reaction to this unexpected heresy was immediate, but the more temperate and reasoned criticisms have only now begun to appear in print.

Miyashiro based his case largely on comparisons between the geochemistry of Troodos rocks and that of recent ocean floor, on the one hand, and between Troodos geochemistry and island arc geochemistry on the other. In particular, he used SiO_2 contents and Fe/Mg ratios to show that the lower pillow lavas and the sheeted complex of Troodos contain a relatively high proportion of rocks with calc-alkalic affinities as well as others which follow

the more expected tholeiitic trends. The significance of this discovery is that, if dredge samples are anything to go by, calc-alkaline igneous rocks of deep oceanic origin seem to be very rare. By contrast, island arc volcanism and calc-alkaline rocks are frequently observed in association. The implication, therefore, is that Troodos (and possibly other ophiolite sequences) is more likely to have been formed in an island arc environment than along an oceanic ridge.

The first serious criticism of this thesis now comes from Hynes (*Earth planet. Sci. Lett.*, **25**, 213; 1975) who argues that rocks of the Troodos complex are so altered that bulk chemical analyses must be regarded as suspect, if not actually worthless, as indicators of origin. Indeed, the extensive alteration of the sheeted complex and the lower pillow lavas has been recorded by many workers since at least 1959 (see, for example, Wilson, *Geol. Surv. Dep. Cyprus Mem. 1*, 1959) and the abundance of secondary silica in Troodos rocks has been a frequent theme. On these grounds, therefore, any direct comparison between the chemistry of Troodos and that of unaltered rock from the ocean floor cannot be valid. To illustrate the dangers of placing too much reliance on chemical data, Hynes goes on to describe the Othris ophiolite suite in Greece which comprises rocks lithologically and petrographically similar to those of Troodos. The chemical characteristics are similar too, which would imply that Miyashiro's arguments are equally applicable. But in the Othris case there is stronger mineralogical, and especially regional geological, evidence in favour of a ridge origin against which the chemical data pale into relative insignificance.

In his reply to Hynes, Miyashiro (*Earth planet. Sci. Lett.*, **25**, 217; 1975) admits that some Troodos rocks have been subjected to secondary alteration but claims that these lie mainly in the upper pillow lava series which he ignored for that very reason. By contrast, rocks in other parts of the complex are "not so unusual in chemical composition". Many such rocks, for example, contain 70–80% anhydrous SiO_2 ; and if this had been brought about by the metasomatic introduction of silica into a basalt containing only 50% SiO_2 , the proportion of material added from outside would have amounted to more than 100% of the original basalt. This, claims Miyashiro, is "not plausible". Primary silicic igneous rocks do therefore exist in Troodos and useful information may be obtained from their bulk chemical compositions.

In brief, this information is that about half of the volcanic rocks in

the sheeted complex and lower pillow lavas of Troodos have $\text{SiO}_2 > 52.5\%$ and $\text{FeO}^*/\text{MgO} > 2.0$ whereas most ocean ridge volcanics have lower values for these quantities. This is "decisive" evidence against an oceanic origin for Troodos but is consistent with formation in an island arc (although it is also consistent with a continental origin). Moreover, Miyashiro says that he "cannot understand" why the geo-

logical information quoted by Hynes supports the existence of accreting plate margins in the vicinity of Mesozoic Greece, arguing instead that this evidence is "so ambiguous" that it could be taken to support either view.

More generally, Miyashiro suggests that the world's ophiolite sequences are so diverse that some were probably created in island arcs, others at oceanic

ridges and yet others in hot spots and stable continents. This point is well taken by Moores (*Earth planet. Sci. Lett.*, **25**, 223; 1975) who nevertheless feels that Miyashiro is wrong about the particular example of the Troodos Massif. Moores notes, for example, that much of Miyashiro's case is based on the premise that calc-alkalic rocks are rare in the ocean floor. But the fact is that most of the rocks obtained from the deep ocean floor have been dredge samples which are predominantly surficial and thus not representative of the oceanic crust as a whole. In this respect, Miyashiro has not been comparing like with like. When a crustal cross section has been sampled (for example, on faults in the Mid-Atlantic ridge and the Ninety East ridge), the rocks have been found to have compositions comparable to those of ophiolite sequences.

On the question of alteration, Moores has re-examined sections of the Troodos rocks analysed by Moores and Vine (*Trans. R. Soc.*, **A268**, 443; 1971) and finds that almost all of the rocks are indeed altered, most of them having abundant vesicles filled with zeolites or silica and many representing greenschist facies metamorphic assemblages. Thus in spite of claims to the contrary, Miyashiro has apparently been citing data influenced by metasomatic alteration. Primary SiO_2 -rich igneous rocks do exist in ophiolites; but they account only for a very small proportion of the total volume and are significantly different from the genuine calc-alkalic rocks found in island arcs, especially insofar as they have much lower K/Na ratios. Moores thus concludes that bulk chemistry is not an adequate basis for differentiating between the products of ridges and island arcs and that recourse must be made instead to structural and stratigraphic arguments.

In a much longer reply to Moores, Miyashiro (*Earth planet. Sci. Lett.*, **25**, 227; 1975) attempts to refute his opponents' arguments in both general and specific terms, on the basis both of "logic and evidence". On the question of ocean floor sampling, he claims (with evidence) that the Ninety East ridge is atypical of oceanic ridges and that there are no grounds for assuming that the mid-Atlantic ridge fault represents a particularly deep section of oceanic crust. At the same time, sampling is not as unrepresentative as is sometimes claimed because rocks formed at depth are exposed along median valley walls and in transverse fracture zones across oceanic ridges. On the question of alteration, he presents additional data to support the view that Troodos compositions cannot be attributed to weathering, metamorphism or metasomatism but arise in-

Anions and membranes

from a Correspondent

THE identification, isolation and partial characterisation of a human erythrocyte membrane protein essential for anion transport (Cabantchik and Rothstein, *J. Membrane Biol.*, **15**, 207-226 and 227-248; 1974; Ho and Guidotti, *J. biol. Chem.*, **250**, 675-683; 1975) provides strong support for Gunn's hypothesis that the various anion exchanges observed in the red cell occur through the passive diffusion across the membrane of a common carrier-anion complex. It is of interest to compare this work with the recent work, particularly that of Narumi's group (*Biochim. biophys. Acta.*, **311**, 80-89 and 90-97; 1973), on the mechanism of HCl secretion by gastric mucosal cells. As part of this mechanism internal bicarbonate is exchanged for external chloride, a situation analogous to that in the red cell, but in contrast to the red cell there is evidence of an active bicarbonate transporting system.

For many years it was thought that simple anions crossed the red cell membrane, diffusively, as free ions and that the differential permeability of the membrane to cations and anions was the result of fixed positive charges within the membrane. This theory was particularly attractive in the light of Bangham's work showing that the permeability of a simple lipid bilayer to different ions could be altered by changing the surface charge of the bilayer. A less attractive feature of the theory was the high activation energy of ion transfer.

This activation energy was closer to those usually associated with active ion transport. When it was shown that the rate of transport of several anions reached a saturation level as the ion concentration was increased the free ion diffusion theory had to be replaced by one involving a carrier ion complex. Cabantchik and Rothstein showed that the carrier was a glycoprotein by treating red cells with a non-penetrating fluorescent marker (SITS), and demon-

strating that sulphate permeability was inhibited when a relatively small population of sites on the external side of the membrane were labelled. They then isolated the label by proteolytic digestion and showed it to be attached to a glycoprotein of molecular weight roughly 9,500. Ho and Guidotti have also isolated a glycoprotein of similar size which is responsible for phosphate transport and identified it with Bretscher's component a. It is tempting to assume that these proteins are identical and are also responsible for the physiologically important chloride-bicarbonate exchange but this has yet to be shown.

As the mechanism of anion exchange in the red cell has become clearer that of the gastric mucosal cells has become more complex. The presence of a bicarbonate ATPase in gastric mucosa, and several other tissues where bicarbonate transport is important, has suggested the presence of a bicarbonate transporting mechanism analogous to the ATP-dependent cation pumps. The demonstration by Namuri and Maki that carbonic anhydrase, which is necessary as a source of bicarbonate ions in gastric mucosal cells, is activated on phosphorylation by a cyclic AMP-dependent kinase would seem to complete the picture by linking the bicarbonate pump to hormones which control gastric secretion and which are thought to act by increasing the level of cyclic AMP in the acid-producing cells. The major problem in this picture is the localisation of the ATPase: most of it is found in the mitochondrial and not the plasma membrane fraction (Soumarmon, Lewin, Cheret and Bonfils, *Biochim. biophys. Acta.*, **339**, 403-414; 1974). But a bicarbonate pump in the mitochondrion would itself be of interest and the isolation of this ATPase as a Triton extract may indicate that a comparison between a passive and active carrier for the same ion will soon be possible.

stead from crystallisation differentiation. Moreover, he suggests that, by involving silica metasomatism for Troodos, supporters of the ridge origin are contradicting themselves insofar as modern oceanic ridges show no signs of metasomatism.

In a final critical article, Gass *et al.* (*Earth planet. Sci. Lett.*, **25**, 236; 1975) point out, among other things, that in discussing the FeO*/MgO ratio Miyashiro has taken no account of the replacement of Mg-bearing mineral phases by carbonates and other non-magnesian pseudomorphs, and that the analyses quoted by Miyashiro were carried out before the precise boundary between the upper and lower pillow lavas was defined (so that many are in fact from the upper lavas). To these specific objections, however, Gass and his colleagues add what is perhaps the most telling general point of all, namely that in this whole argument there has been more than a tendency to ignore the wide variety of evidence available.

The fact is that few, if any, workers have claimed that an oceanic origin for Troodos has been proved. Instead, the case is based on an extremely wide range of evidence from many different branches of the earth sciences, with no single piece of evidence yet obtained being regarded as conclusive by itself. By concentrating public disagreement largely on the geochemical data, support for Troodos as ancient ocean floor has thus somehow been made to appear more equivocal than it really is. For the time being, the balance of argument remains with Miyashiro's opponents.

Rotating fluids

from a Correspondent

'Can the geomagnetic dynamo be powered by precession?' was one of the geophysically motivated questions raised at the recent Euromech meeting entitled "Non-linear processes in rotating fluids" held at University College, London between April 14 and 17.

THE sufficiency of precession as an alternative power source to convection in the Earth's core was discussed by Jacobs (University of Cambridge). Estimates of the power required to maintain the geomagnetic dynamo against ohmic dissipation have been variously assessed as between 10^9 and 10^{12} W with the probable value between 10^{10} and 10^{11} W. According to Jacobs and his co-authors (Rochester, Smylie and Chong) the precessional power input to the core (at the expense of the obliquity) fails by at least two orders of magnitude to satisfy this require-

ment, and thus the possibility of a precession-driven dynamo is extremely remote. This contrasts with the views of Malkus (*Science*, **160**, 259; 1968) and, more recently, Stacey (*Geophys. J.*, **33**, 47; 1973) who have both argued in favour of precessional torques producing the driving mechanism for dynamo action. Jacobs attributed the disagreement not only to Malkus's failure to allow for electrical conductivity contrast at the core-mantle interface and Stacey's incorrect determination of the tilt-over angle, but also to the neglect by both authors of the dependence of the strength of the dissipative part of the core-mantle coupling on the diurnal frequency of the precession-induced core flow relative to the mantle. Loper (Florida State University and University of Newcastle upon Tyne) was equally pessimistic regarding the role of precession. His investigation of the transmission characteristics of the core's hydromagnetic boundary layers indicated again that precessional power is inadequate by at least 10^2 .

In attempting to extend further the laboratory modelling of atmospheric flows in the rotating annulus, Leach (Meteorological Office) investigated the particular effects of topography and found that its presence significantly affected the well-known transitions between the different flow regimes and that it reduced the amplitude of the free baroclinic waves. The interplay between these free waves and those generated by the topography resulted in an increase in heat transfer, indicating that the free waves play a diminished part in the energetics of the system. A related study by Mason and Hide (Meteorological Office) into the effects of sloping end walls upon the transition from axisymmetric flow to baroclinic waves was illustrated by an interesting cine film. Various combinations of end-wall orientations were investigated and non-linear effects were identified by comparing the observed wave numbers with those predicted by appropriate linear theory.

The meeting also included contributions from other branches of fluid mechanics. Wimmer (University of Karlsruhe) reported a very careful experimental investigation into the viscous flow between rotating concentric spheres (the outer sphere usually being stationary). Measurements of the friction torque were presented for a wide range of gap widths and Reynolds numbers. Photographs of the transitions from laminar stable flow to unstable flow to turbulent flow were shown and it was stated that, in addition to the well known Taylor-Görtler-type vortices, several new types of instability were discovered. One example in the wide gap case was a class of instability confined to the equator (the

number of vortices being independent of Reynolds number) and having five different modes of instability for the same Reynolds number.

Several numerical investigations into atmospheric phenomena and related laboratory models were presented, including two papers by Hoskins (University of Reading) and Simmons (University of Reading) on non-linear properties of baroclinic waves and the formation of fronts, and the results of Quinet (Meteorological Institute, Brussels) on a numerical simulation of amplitude and tilted-trough vacillation observed in the annulus experiments. Egger (University of Munich) reported the results of his numerical experiments on the cyclogenesis occurring in the lee of a long north-south mountain chain as this barrier is approached by a depression. By studying the time-development of the system it was claimed that a satisfactory mechanism for the cyclogenesis process could be obtained.

Space tribology

from a Correspondent

The first symposium in Europe, if not in the world, to be devoted to space tribology was organised by the European Space Research Organisation at its establishment at Frascati on April 9, 10 and 11.

THE papers varied from a very fundamental contribution by M. Barquins, R. Courtel and M. Maugis (Centre National de la Recherche Scientifique, Meudon) on the friction of Cu-Cu, Mo-Mo, Cu-Mo and Cu-Ni couples in ultra high vacuum to a paper by E. J. Robbins (European Space Tribology Laboratory, Risley) on the testing of the tribological performance of space mechanisms in a vacuum, in which the author presented a clear picture of the true environment in the satellite and considered the effect of water and other desorbed vapours on spacecraft mechanisms.

In general the emphasis was upon dry lubricated systems and a whole session was devoted to lead film lubrication of rolling element bearings, which is a largely European development, with now over 10^6 hours of vacuum operation to support it. The limitations of the performance of the film in air were clearly indicated in a paper by M. J. Todd (National Centre for Tribology, Risley) who reported tests showing rapid bearing failure at speeds beyond about 20 r.p.m.

Another session was devoted to the performance of slip rings in ultra high vacuum, mostly operating in the dry lubricated regime. The behaviour of slip rings under these conditions is sur-

prisingly good, both from the point of view of low noise and low wear, and it is now possible to use them for mechanisms requiring lives of 6 to 7 years in space. The four papers in this session show that the European and US approaches are very similar, both using Ag.Cu.MoS₂ compact brushes on silver slip rings. A paper by W. Wilkens (DFVLR, Braunschweig-Flughafen) presented some new data on the performance of dry lubricated slip rings moving at very low speeds, such as are encountered on solar panel drives on communication satellites.

The only paper presenting work on gears in UHV came from E. Conde who reported some of the results of a very extensive programme at the CNES establishment in Toulouse. There seems to be no other work on the performance of gear pairs in UHV in progress in Europe.

One of the most important tribology problems for long life spacecraft is the provision of a bearing-lubricant system for momentum wheels. These units normally rotate at 3,000–4,000 r.p.m. and must perform reliably for 7 to 10 years in space. All momentum wheels flown to date are mounted on ball bearings with controlled minimum lubrication systems. W. Auer of Teldix, Heidelberg, presented a paper on the development of a European momentum wheel which is now flying in the Symphonie satellite and which embodies a lubricant store that progressively releases its oil to the bearings.

A unique and very interesting development in Europe is the application of grease lubricated hydrodynamic bearings to momentum wheels. Philips of Eindhoven have pioneered this work in the world and E. A. Muydermann and U. van der Wal presented two papers which discuss both the principle of the design and the present state of development. Nine bearing systems have each now completed more than 44,000 hours operation under various conditions of load and environment and the authors are now confident that all problems of shear instability and retention of the grease have been solved. The wheel has been chosen to fly in the ESRO Orbital Test Satellite now under construction.

Also in the field of liquid lubrication was a paper by G. C. Friend. A. J. Grosiek, W. A. Ware and M. Warwick introducing four new space lubricants developed by BP Research Centre. Two of these are fine cut oils of very low vapour pressure, one a petroleum oil and the other a di-ester, which are available specially filtered in small quantities. The other two are greases based upon the oils but using oleophilic graphite to perform the dual role of boundary lubricant and thickener.

These lubricants are now undergoing life tests.

A very important paper by B. Baxter (British Aircraft Corporation, Stevenage) presented the results of a physical-chemical examination of the degradation of a grease which had run in a bearing for some 14,000 hours in vacuum. The author has developed at BAC a technique for examining the interaction of bearing and lubricant, which in this case was able to show that the 12 hydroxy-stearic acid in the grease-thickening agent is lost during running in low oxygen conditions. The technique is extremely valuable in gaining deeper insight into the reasons for bearing-lubricant system failure and the same author employed it very successfully in 1972 to explain the well known but poorly understood formation of 'brown sugar' in miniature bearings. The use of this technique in space tribology is very new but it can be expected to make significant contributions in the future.

The need for some harmonisation of the space tribology work in Europe was stressed by H. M. Briscoe, ESRO, in his closing address. ESRO is partly able to perform this task by acting as a clearing house for information and as adviser on future requirements, but he suggested that a small international advisory committee might be more effective at national level in preventing unnecessary duplication.

Crystallisation and chain extension in polymer solutions

from Paul Calvert

IN recent years the study of morphology in crystalline synthetic polymers has reached an impasse. Twenty years ago the discovery that 10 nm, plate-like crystals could be grown from solution led to the development of the folded chain model, by Keller and others. Now, thousands of man-years of research and several hundredweight of publications later there is still little hard evidence as to whether the chains fold tightly or loosely and whether re-entry is adjacent to the point at which the chain leaves the crystal or whether it is at random. One way to try to gain more understanding is to look at the effect of aligning the chain before crystallisation. Mackley and Keller (*Phil. Trans. R. Soc.*, **278**, 29; 1975) describe such a study of molecular orientation and crystallisation in flowing polymer solutions.

In 1965 Pennings and Keil (*Kolloid Z.*, **205**, 160; 1965) described fibrous

'shish kebab' crystals, grown from stirred polymer solutions at high temperatures where unstirred solutions would not crystallise. These structures have been studied in detail by Pennings *et al.* (*Kolloid Z.*, **235**, 99; 1970; **237**, 336; 1970) by Krueger and Yeh (*J. macromol. Sci. (Phys)*, **B6**, 431; 1972) and by others. The shish kebab, of overall diameter about 1 μ m and length up to several mm, apparently has a central core, the 'shish', about 20 nm diameter skewering lamellar crystals, the 'kebabs'. The shish and kebab are however molecularly connected, not distinct. It was found that turbulence was necessary for their formation, probably because this resulted in elongational as opposed to shear flow and so straightened the dissolved molecules.

Mackley and Keller give a detailed description of results obtained with an apparatus designed to produce pure extensional flow, first described with Frank (*Polymer*, **12**, 467; 1971). Two opposed jets of solution give extensional flow in the symmetry plane when both blow and on the common axis when both suck.

Calculations for dissolved polymer chains suggest that there should be a rapid increase in molecular extension at a critical flow strain rate, which is molecular weight dependent. Observations on the appearance of reversible birefringence in solutions above their crystallisation temperature support this and suggest that molecular alignment is almost complete. It should be noted though, that 95% alignment as measured by birefringence could still correspond to one sharp bend for every twenty chain units, so that the molecule is locally aligned yet compact in overall shape, like a pack of sausages. It would be helpful to have more information on molecular shape, such as a light scattering study might provide.

As the temperature is lowered fibrous crystallisation takes place in the high strain-rate regions of flow. The maximum temperature for crystallisation increases with strain rate, concentration and molecular weight as might be expected if the flow elongates the molecules, reduces their entropy and so increases the effective dissolution temperature. It should be possible to see crystallisation under flow temperatures where the crystals redissolve when flow stops.

The most remarkable morphological observation is that shish kebabs grown under extensional flow do not look any different from those grown from turbulent stirred solutions. Several models for the formation of these structures can be envisaged. Mackley and Keller seem to favour formation of an extended chain shish nucleus on which folded-chain lamellar kebabs sub-

sequently grow. Alternatively the shish kebab could be a development of the stacks of polymer crystals formed from static solutions, where the stacks resemble either an open book or a spiral staircase. If the gaps between crystals were opened up these would look like shish kebabs. Nagasawa and co-workers (IUPAC Symposium on Macromolecules, Aberdeen, 1973) reported the formation of shish kebabs by deformation of single crystal stacks and suggest that the shish kebab structures produced from flowing solutions are also the product of the mechanical disruption of stacked single crystals by the fluid flow. This idea would explain the similarity of structures produced under different conditions, the amount of connecting material between kebabs and the general irregularities of shish kebabs. This question could be resolved by studies of how shish kebabs grow.

Thus this elegant work will add to our understanding of flow in polymer solutions and thermodynamic aspects of crystallisation but tells us little about the crystallisation process. Methods are still needed to study the molecular arrangement in these structures. Results on crystallisation of polymer melts in the same apparatus should be as interesting and more directly relevant to polymer processing methods such as fibre spinning. In this respect this simple, controllable crystallisation system could be very enlightening.

Halley lecturer produces new theory of comet origins

by John Gribbin

ON May 6 the 1975 Halley Lecture was presented in Oxford by W. H. McCrea, Professor Emeritus of the University of Sussex. McCrea took as the title of his discourse "The Solar System as a Space Probe", a far-ranging topic which suggests both an explanation of ice ages and a model for the origin of comets.

As McCrea pointed out, the advent of the space age has renewed interest in the study of the Solar System, but in many ways the Solar System itself is the best space probe of all, travelling at present towards a point in the constellation Hercules. The speed of the Solar System — 20 km s^{-1} relative to the rest frame of local stars; 140 km s^{-1} relative to the so-called 'high velocity' stars — may seem small compared with the speeds that are necessary to cross the Galaxy in a reasonable time, by human standards. But the great age of the Solar System, some 5×10^9 years, suggests that even at these modest speeds the system has

already orbited around the Galaxy 20 times. So there has been ample scope for samples to have been gathered from different regions of the Galaxy.

The most obvious features of spiral galaxies, like our own, are the spiral arms (made up of bright stars) and the associated dark lanes of gas and dust. Can the passages of the Solar System through these features have left traces for us to discover? McCrea suggests that they have. The spiral arms are thought to be a more or less permanent feature, with the dark lanes along their edges showing the formation of shock waves as clouds of gas run into the arms.

In our Galaxy, there are two main arms, and in round terms the time scales associated with the motion of the Solar System produce a passage across one arm every 100 Myr. It takes 10 Myr to cross each arm, and 1 Myr to cross the lane of shocked and compressed clouds at the edge of the arm. Now, the Sun is just entering the Orion arm, having recently passed through the compression lane which lies just inside that arm.

Following in illustrious footsteps (including those of H. Shapley in 1920 and 1949, F. Hoyle and R. Lyttleton in 1939) McCrea suggests that it is just such passages of the Solar System through the dark lanes which bring about ice ages. Details of this new variation on an old theme are shortly to be published in *Nature* (in our issue marking the 300th anniversary of the foundation of the Royal Greenwich Observatory).

But the astronomers among McCrea's audience really began to sit up and take notice when he moved on to discuss the problem of the existence of comets. These enigmatic objects, with masses in the range 10^{16} – 10^{18} g, are the least massive members of the Solar System (except for small asteroids) but in many ways the most baffling. Have they always formed part of the Sun's family, or are they ephemeral visitors snatched from the depths of space? Most theorists seem to lean towards the former view; but McCrea has now come up with a model which might change that consensus.

The most popular concept of a comet (but not the only model) sees it as a 'dirty snowball' of water ice and silicates. As McCrea notes, this composition is much the same as the best available models of interstellar grains, which are now regarded as silicates covered by ice. So, if a ball of interstellar dust could be separated out from the gas, we would have something very much like the nucleus of a comet. The snag is that the presence of the gas will tend to keep the interstellar grains apart even in a

situation where with no gas they would congregate under their mutual gravitational attractions.

McCrea gets round this by suggesting that dust can collapse independently of the gas if the mean free path of a gas molecule (relative to dust grain encounters) is longer than the minimum radius of a cloud of dust at the same density which would collapse if free from gas. Putting in the numbers, he comes up with a range of masses for the resulting aggregations, and these are just in the range of comet masses. For example, grains $0.25 \mu\text{m}$ across will form aggregates with mass about 1.6×10^{18} g. But to set the whole process off, the gas-dust mixture must be compressed to densities rather greater than the typical density of matter in interstellar space.

Once again, this is where the interaction of the moving gas with the spiral arms comes into the picture.

'Normal' clouds, says McCrea, have densities of about 0.1 – 10 molecules cm^{-3} . Dense clouds have about 10^5 – 10^7 molecules cm^{-3} over regions one or more parsecs across, and the compression lanes must be at least as dense as the dense clouds. When a normal cloud hits a compression region, the first product is likely to be a dense cloud. At successive further compressions as the cloud continues its journey around the Galaxy and across spiral arms first cometary nuclei, then stellar and planetary condensations and even clusters of main sequence stars could be formed, depending on the starting density of a particular cloud. On that picture, the comets which now form part of the Solar System are a by-product of the recent passage through a compression lane, captured by the Sun en route. It may be that we are lucky to see any comets at all, and that for most of the long history of the Solar System there have been no comets bound to the Sun. Those we have at present will soon be dispersed, or broken up by repeated passage into the inner Solar System and past the Sun.

All this is dramatic enough. But the implications of the quite profound changes on Earth which occur as the Solar System moves through different regions of the Galaxy go still further. These "cosmic seasons", as Shapley dubbed them, could well have influenced the development of life, which (in the form we know it) may depend not just on the distance of a planet from its star, but also on the distance of the star from the galactic centre. McCrea terminated his lecture with the comment that the effect of all these astronomical influences on life "can be very complicated".

review article

Mutation selection and the natural history of cancer

John Cairns*

Survival of the rapidly renewing tissues of long-lived animals like man requires that they be protected against the natural selection of fitter variant cells (that is, the spontaneous appearance of cancer). This article discusses three possible protective mechanisms and shows how they could explain various features of the natural history of certain common cancers of man.

We are accustomed to thinking of the combination of natural variation and natural selection as a force for the good, that creates and maintains the fittest in a species and discards the unfit. This is the fundamental theorem of biology. But when we turn from the competition between the individuals of a species to the competition between the individual cells within a single animal, we see that natural selection has now become a liability. The dangerous mutations are now those that confer on a cell an increased survival advantage. We may therefore expect to find, especially in animals which undergo continual cell multiplication during their adult life, the evolution of mechanisms that protect the animal from being taken over by any "fitter" cells arising spontaneously during its lifetime—that is mechanisms for minimising the rate of production of variant cells and for preventing free competition between cells.

The cells of the surface epithelia that separate a typical mammal from its environment undergo continual turnover, and this turnover accounts for most of the cell division that occurs in the life of the animal. For example, a newborn rat contains some 3×10^9 cells and grows to a final value of about 6×10^{10} cells in old age; excluding any programmed cell death, the rat therefore represents a total of 6×10^{10} cell divisions. During its entire lifetime, however, the rat produces and discards about 10^{13} epithelial cells from its small intestine alone, and almost as many cells from various other epithelia¹. Its risk of being overwhelmed by variants is, in principle at least, related to this total of 10^{13} . For man, who lives ten times as long and is one hundred times as large, there must be some 10^{16} cell divisions, and this number must somehow be reconciled with a spontaneous mutation rate of about 10^{-6} per gene per cell division²⁻⁴. Because most of the cell division is occurring in epithelia, that is where we may expect to find the protective mechanisms most highly developed.

Our interest in these protective mechanisms arises not least of all from the fact that they will determine how "fitter" variants are created by mutagenic agents and how such variants can be helped to supplant their normal neighbours (the process of carcinogenesis). Here we come to an added reason for concentrating on epithelia. Although most experimental cancer research, especially that using oncogenic viruses, deals with

tumours of supporting tissues and blood-forming cells (the sarcomas and leukaemias), human cancer is predominately a disease of the various surface and glandular epithelia (Table 1).

This paper describes various ways in which the programme of cell renewal in epithelia may have evolved to minimise the risk from somatic mutation, and finally how certain features of natural carcinogenesis could be explained in terms of such a programme.

Lineages and the place of stem cells in programmes of cell renewal

The turnover that occurs in the self-renewing epithelia is the result of continual shedding of superficial cells balanced by continual multiplication of the deeper cells. In the simplest examples like skin, cell division is restricted to the deepest (basal) layer of cells. To keep the number of basal cells constant, one of the two daughter cells resulting from each cell division must on average remain in the basal layer and the other must escape and be discarded. Obviously there are several possible programmes of cell division that could produce this result: a regular programme of asymmetric division, where one of each pair of daughter cells is invariably exported (Fig. 1a); or a random choice of cells for export, the rule being simply that the available space for basal cells should be kept fully occupied; or lastly some more complex programme in which cells destined for export are allowed to undergo a certain number of divisions before they are exported from the basal layer (Fig. 1b). Some elegant experiments on the fate of pairs of daughter cells, arising in the rat oesophagus, suggested that the choice was random⁵. It now seems more likely, however, at least in skin^{7,8} and small intestine⁹, that a fairly rigid programme is operating of the kind shown in Fig. 1(b)—that is, most of the dividing cells produce descendants that are all ultimately exported.

An arrangement of this kind will decrease the rate of accumulation of spontaneous mutations and the risk from exposure to mutagens. The only mutations that accumulate during the life of an animal are of course those that arise in cells that themselves survive or contribute descendants that survive for the animal's lifetime—that is, the mutations must occur in the "immortal" stem cells (drawn as squares in Fig. 1). Any muta-

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tion that arises in a cell destined to be discarded will be lost (unless of course its effect is expressed quickly and serves to prevent the cell from being discarded).

The smaller the proportion of cells that are immortal stem cells, the lower the risk. In the different self-renewing systems of mammals the proportion probably ranges from one in ten (mouse skin)⁸ to one in several thousand (bone marrow)¹⁰. But in most instances the number of immortal stem cells is not known. Note, however, that it may be much less than the number of cells capable of being promoted to immortality by force of circumstances (for example, that are capable of recolonising an epithelium or bone marrow depleted by X irradiation) or the number of cells that are still undifferentiated (the usual, but in this context less useful, meaning of the term "stem cell").

Stem cell division and the segregation of old and new DNA strands

Although the division of each immortal stem cell can produce two apparently indistinguishable daughter cells the fate of which may, in many systems, be determined simply by their position in space, it does not follow that the two daughters must be genetically equivalent and must therefore be equally likely to contain mutations.

Most spontaneous mutations probably arise as errors during DNA replication. Because DNA is duplicated semi-conservatively, these mutations are present initially in "heterozygote" molecules with one mutant strand and one normal strand. Unless such a heterozygote molecule is converted to homozygosity by one of the DNA repair mechanisms of the cell it will, on subsequent duplication, yield one normal daughter molecule and one molecule which is mutant in both strands (Fig. 2a). We see at once that stem cells would be protected against errors

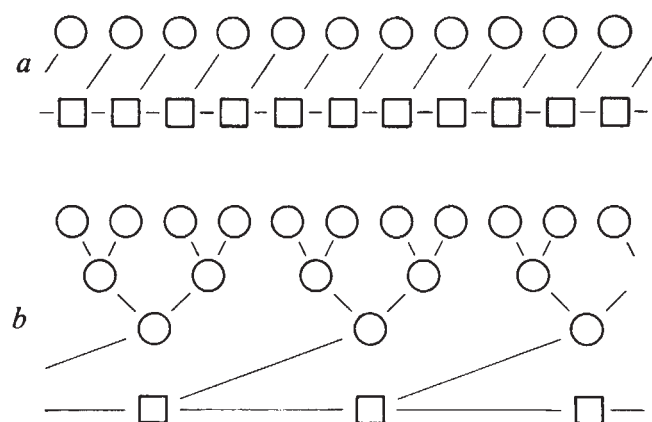


Table 1 Cancer incidence in Denmark, 1943-67

Type	Commonest sites	Total cases	%
Carcinomas			
External epithelia	Skin, large intestine, lung, stomach, cervix	168,591	56
Internal epithelia	Breast, prostate, ovary, bladder, pancreas	110,182	36
Sarcomas and leukaemias		23,801	8
TOTAL*		302,574	100

These figures are drawn from three reports that together summarise the experience of the 4.5 million inhabitants of Denmark over a period of 25 years (ref. 5). They were chosen here because, unlike most national registries, the Danish Cancer Registry reports separately for each site the number of carcinomas arising in the epithelial (parenchymatous) cells and the number of sarcomas arising in the supporting cells.

* This total excludes about 10,000 cases in which the exact site of the primary cancer was not known.

requires that each stem cell must enter mitosis with its duplicated chromosomes correctly aligned with respect to its surroundings. Up to a point this is certainly true. For example, in skin the plane of division of the basal cells is so arranged that both daughter cells initially lie side-by-side in the basal layer; further, there is some evidence that the successive mortal daughters of each stem cell lie alternately on one side and then on the other side of an unchanging plane of division⁸. In fact, such a pattern would fit nicely with the observation that when a given chromosome is followed through repeated rounds of duplication, in a cell forced to undergo endoreduplication by being grown in colchicine, each parental strand is found to have segregated first to one side of the plane of division and then to the other (Fig. 2b)¹¹.

Fig. 1 Two possible programmes for the renewal of an epithelium by multiplication of the cells in the deepest (basal) layer, *a*, Renewal simply by regular, asymmetric division of stem cells; *b*, renewal by asymmetric division of the stem cells, plus some further division of the cells destined to be exported to the surface. The immortal stem cells are shown as squares and the mortal, differentiating cells as circles.

of duplication if it were so arranged that the immortal daughter cell always receives the DNA molecules which have the older of the two parental strands (that is, the rightmost molecules in Fig. 2a) and the mortal daughter always collects the molecules with the younger parental strand (that is, always collects the mistakes made in the previous generation). This rule would therefore maintain an "immortal strand" through successive generations.

To operate such a rule, the immortal strands must all be marked in some way so that sister chromatids can be distinguished at the centromere, and all centromeres must behave in a coordinated fashion. Also, if the rule is to apply to all parts of the genome and not merely to the centromeric regions, the cell must ensure that sister chromatid exchanges are rare.

In addition, if the fate of the two daughters of any immortal stem cell is determined by their position in space, the rule

Unlike the matter of stem cell lineages which is reasonably well understood, this speculation about the relationship between stem cell lineage and DNA lineage is completely unsubstantiated. In many instances, cells labelled in their DNA during one cycle have been shown to divide this label unequally among their grand-daughters¹², and there is one example of a cell with several chromosomes (the germinating spore of *Aspergillus*) which regularly segregates the DNA strands in these chromosomes as if they are parts of a single DNA duplex¹³. It remains to be shown, however, that epithelial stem cells behave in this way. The test, of course, is to see whether stem cells can be permanently labelled in their DNA at the time they are first formed (that is, can be shown to have a DNA strand that is immortal) or alternatively to see whether they retain the label only for one generation when labelled in an adult animal (that is, normally discard the newer of their

parental DNA strands). Experiments of this kind are not yet completed.

Compartmentalisation and the natural selection of fitter variants

Having discussed two arrangements that would reduce the effective mutation rate in multicellular animals, we can now turn to a factor influencing the selective forces operating on mutant cells.

Most experimental cancers are formed only after repeated or prolonged exposure to a carcinogen. Similarly, most human cancers appear in old age, as if they usually arise as the end result of a lifetime of carcinogenesis. For this reason it is generally believed that several steps are required to convert a normal cell into an uncontrolled, invasive variant. The exact number of steps could be determined from the relationship between age and incidence provided that all steps occur at the same, unchanging rate^{14,15}. If, however, the first step increases the survival of the altered cell so that this cell type steadily increases its number at the expense of normal cells, there will be a corresponding steady increase in the chance that somewhere there is a cell undergoing the next step, simply because with time there will be more and more first-step cells at risk^{16,17}. So, whatever the number of restraints that must be overcome to convert a normal cell into a cancer cell, the risk that this occurs will be much increased if there are intermediate states with high survival advantage and if these variants are in fact able to displace their neighbours. We may therefore expect to find fast-multiplying tissues arranged in such a way that neighbouring stem cells (or sets of stem cells) are restricted to limited territories so that they cannot easily compete with each other.

The epithelium of the small intestine, which is the site of the most rapid cell turnover in the body, is an example of just such an arrangement. Cell multiplication is confined to indented regions of epithelium (the crypts) that project deep into the underlying mesodermal tissues. Between each neighbouring pair of crypts the epithelium forms a fold (the villus) that projects into the lumen of the gut. As a result of the continuous cell division in the crypts there is a continuous flow of cells from each crypt to the tips of the neighbouring villi, and from there the cells are shed into the gut¹⁸. Apparently each cohort of cells moves *en bloc* up the villus, propelled by the pressure of new cells being formed in the crypt. No matter how many immortal stem cells are normally present in each crypt (and the number is not known), any variant stem cell with high survival advantage should quickly exclude normal cells from its crypt. Unless this variant is, however, able to alter the whole structure of the epithelium (for example, by invading the surrounding meso-

derm) the only way it can reach neighbouring crypts is by moving down the other sides of the villi, against the continual flow of normal cells arising in these crypts.

We see therefore that the arrangement of the intestinal epithelium into crypts and villi will tend to limit the expansion of any population of fitter variants that might otherwise form an ever-increasing target for further mutational steps. Other epithelia are no doubt partitioned in other ways. In each case the rules governing the territoriality of the proliferating cells will determine which kind of variants are the most successful.

Mutation selection and carcinogenesis

Restricting the number of stem cells, imposing a special pattern of segregation of DNA strands, and restraining the opportunities for competition between cells, are just three of the possible ways that may have been evolved in long-lived multicellular animals for diminishing the rate at which mutant cells accumulate. Of course, the rate would be further decreased if stem cells have unusually error-free forms of DNA synthesis and repair, perhaps in conjunction with a prolonged interval between successive divisions to allow ample time for any necessary repairs. The object of this article, however, is not to produce a list of all the factors that might affect mutation rate, but to show how the organisation of the self-renewing tissues of multicellular animals may often determine the sequence of events during carcinogenesis.

We have seen that the lineage of epithelial cells is so arranged that rapid renewal is normally achieved by rather few divisions of a special minority population, the immortal stem cells, which have a somewhat protected position deep in the epithelium and may, in addition, retain specially preserved DNA strands. The rate of accumulation of mutations in such a system will be proportional to the rate of division of the stem cells or perhaps, more specifically, to the rate at which they have to multiply to replace lost stem cells (that is, the rate at which new immortal strands have to be created). Anything that accelerates either of these processes could be carcinogenic. For example, cigarette smoking causes a thickening of the bronchial epithelium and obviously disrupts the ordered programme of cell renewal^{19,20}. Judging from the relationship between incidence and age and duration of smoking²¹, it seems that the smoker accumulates in each year about the same risk that 50 non-smokers accumulate collectively; alternatively, since incidence increases as the fourth power of time, it could be said that the smoker's bronchial epithelium goes through time at about 2.7 times the normal rate. It is tempting therefore to ascribe the increased risk of lung cancer simply to a 2.7-fold increase either in the rate of division of stem cells in the bronchial epithelium or in the rate at which

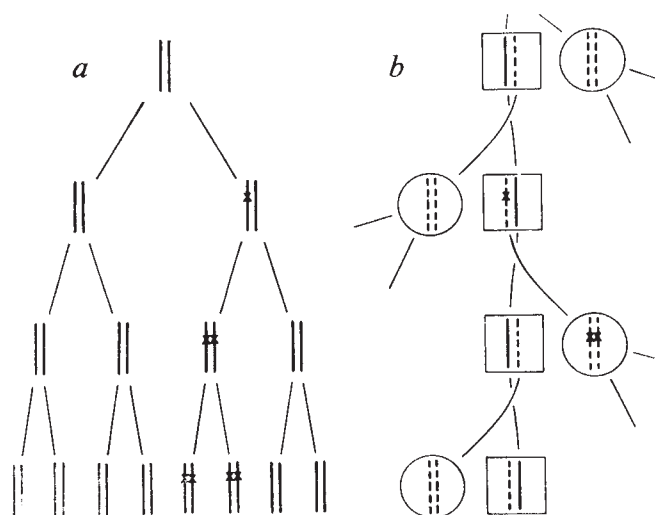


Fig. 2 The lineage of DNA undergoing semi-conservative replication. *a*, The segregation of a mutation, X, that arose as a copying error during duplication. *b*, Rearrangement of this lineage to fit the observation that parental strands apparently tend to segregate alternately to the right and left of the plan of division²¹ and that, in one instance at least, the stem cells also move at division alternately to one side and then to the other⁸. The hypothesis therefore is that stem cells (□) keep the same parental DNA strands through successive cycles.

these stem cells have to be replaced. This example shows that although it is customary to think of carcinogens as necessarily acting directly as mutagens, some could act indirectly simply by altering the programme of division of immortal stem cells.

Nevertheless there is no escaping the fact that the most powerful experimental carcinogens are intense mutagens. So we should now consider what general classes of mutation are likely to lead to cancer. In particular, because most forms of human cancer seem from their age-incidence to be the end-result of several consecutive, independent steps (mutations) we should be especially interested in those mutations that are likely to affect the subsequent rate of formation and selection of additional mutations. The previous example showed how a relatively slight change in the rate of stem cell division may have a great effect on cancer incidence. Obviously the same effect could be produced by an increase in the mutation rate per cells division, resulting from change in a gene with a product concerned with the duplication or repair of DNA. Unless such mutagenic mutations confer some survival advantage, however, they will remain confined to the stem cells in which they arise, and so only if they produce enormously increased mutation rate will they raise significantly the overall mutation rate; for a single cell to double the probability that an organ with 10^8 stem cells has experienced an event which increases as the fourth power of time, that cell would have to move through time (that is, collect mutations) at 100 times the normal rate. Probably more important, therefore, are mutations that affect the interactions of a cell with its neighbours. Any mutation that gives a stem cell the ability to move out of its compartment in an epithelium may cause it to form an expanding clone of stem cells, and because such a clone would be the site of an unusually rapid rate of formation of immortal strands it should show a much higher mutation rate than the rest of the epithelium; the first mutation would therefore be acting as a 'primer' for whatever succession of further alterations are needed to make a fully invasive cancer. The most carefully studied precursor states for any human cancer are the various precancerous abnormalities of the cervix, and they show exactly the properties we would expect of primer mutations. They are characterised by the appearance of extensive clones of cells^{22,23} with deranged programmes of cell renewal^{24,25}; further, although for obvious reasons the undisturbed natural history of these states is not well quantitated, it does seem that the successive levels of abnormality succeed one another with a rather high probability^{26,27}, as if the first level of abnormality has indeed raised the rate at which mutant cells accumulate.

If stem cells do preserve an immortal DNA strand through successive divisions, the only time when it should be easy to create a primer mutation (that is, immortalise an error) may be when these immortal strands are being synthesised—that is, when the epithelium is being created or is having to regenerate. It is therefore significant that carcinoma of the cervix usually arises immediately next to the boundary with the cervical canal, where the stratified epithelium of the cervix changes to the columnar epithelium of the canal²⁸. The exact position of this

boundary is known to fluctuate, and it is easy to imagine that the epithelium here is especially at risk because the local population of each kind of stem cell (stratified and columnar) has to rise and fall with each fluctuation.

Most epithelia probably establish their stem cells *in utero*²⁹, and so, if undisturbed, are unlikely to form primer mutations. One obvious exception, however, is mammary epithelium which proliferates after puberty with each ovarian cycle^{30,31} and further proliferates during each successive pregnancy. In this special case a most interesting relationship is found between the extent of the proliferation that occurs during sexual maturity and the probability that cancer develops in old age³². The overall (lifetime) risk of cancer developing in childbearing women seems to be linearly related to the length of the interval that elapsed between puberty and the first full-term pregnancy, rising in value from 30% to about 130% of the value for women who never have children. So there are two unusual features to be accounted for: although the mammary epithelium proliferates at each successive pregnancy, it is only the first pregnancy that affects the risk of breast cancer; second, although the incidence of most cancers (this one included) rises as about the fourth or fifth power of age, the total lifetime risk of getting this cancer proves to be directly proportional to the length of one particular time interval. The explanation could be as follows. First, we suppose that the stem cells of breast epithelium increase in number at the time of puberty and then undergo a certain fluctuation in number with each ovarian cycle until the first pregnancy, whereupon the population builds up to a final level that is no longer influenced by the ovarian cycle or by additional pregnancies. Second, we suppose that the chance of a primer mutation occurring is simply proportional to the total number of times that immortal strands have had to be made in this epithelium (that is, the number of stem cell generations that have occurred)—much in the way that the frequency of mutants in a bacterial chemostat increases linearly with time³³. Once pregnancy establishes the final unchanging population of stem cells, the epithelium becomes as protected against mutation as any other proliferating epithelium.

For obvious reasons, experimental carcinogenesis is usually studied in systems where brief exposure to a carcinogen produces a high incidence of cancer within a few weeks or months. It could be argued that such systems, using very strong carcinogens, are not suitable models for human cancer which typically arises at a slow rate after an incubation period of many years. Therefore this article, being on the natural history of cancer, has used examples of the common human cancers. The idea that special primer mutations are the first step in the formation of most cancers comes in large part, however, from certain reports of experimental carcinogenesis where the process could be divided into the separate stages of 'initiation' and 'promotion'^{34,35}.

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article

Recent African rainfall patterns

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We present the results of a pilot study aimed at creating a more concrete basis for discussion of the variability in both space and time of rainfall in the Sahel.

FOR the purpose of this report we define the Sahel region as that running across the entire African continent between 10°N and 20°N. The area west of approximately 30°E is a fairly regular plain; to the east are the Nile River basin of Sudan and the mountains of Ethiopia.

The atmospheric circulation system that brings rain to this region is the ascending branch of the tropical Hadley cell, often called the Intertropical Convergence Zone (ITCZ), which moves north of the equator in the Northern Hemisphere summer. This system usually reaches its most northerly position in August so that this is the wettest month over the entire broad region of the Sahel¹. We have concentrated on August for the African region for the years of good climatic records, 1941–73.

The data for this study were derived from the published monthly records² and a magnetic tape of global surface data before 1971 furnished by the National Center for Atmospheric Research. Meteorological agencies of the United Republic of Cameroon, Ethiopia, the Republic of Ghana and the Republic of Mali provided supplementary data.

Figure 1 illustrates the average rainfall patterns for August for the African region for the periods 1941–50 and 1951–60. The stations which usually report are indicated by dots. A similar map for the 10-year period 1961–70 has not been produced at this time because of important gaps in reporting. Several features of these maps are important. The Sahel region had about 10% more rainfall in 1951–60 than in the earlier decade; the centre of the maximum precipitation was approximately 1° farther south in the earlier decade than in the latter; the August rainfall belt across approximately 15°N has two distinct regions, one covering the large plains in western and central Africa and the other in the proximity of the Nile basin and Ethiopian highlands; and there are two areas of orographic rainfall suggested by the small areas averaging more than 600 mm of precipitation for the month.

In Fig. 2 we show analyses of the August precipitation data for 1964, 1968 and 1972. That for 1964 shows a month of relatively heavy rainfall over the Sahel. The pattern is quite similar to those of 1951–60; 1968, on the other hand, is the year in which the belt of maximum precipitation underwent the smallest northward displacement in the 33 yr of adequate record, to only about 7.5°N, compared with the average of about 12.5°N for 1951–60. Nevertheless, August 1968 was not the driest recorded in the Sahel, which suggests that the meridional gradient of precipitation was flatter in this month than in other drier months. Another indication of the uniqueness of this month is the region of heavy, flooding rainfall along the normally dry southern coast between 10°W and 0° longitude³.

The years 1972 and 1973 were those of lowest precipitation in 1941–73 in the Sahel. This is clearly indicated by the break in zonality of the 200 mm isohyets along 15°N in the map of August 1972.

Figure 3 illustrates a relatively crude attempt to show meridional 'cross sections' of the rainfall pattern across the Sahel, derived from a series of eight stations along approximately 0° longitude, for August of 1964, 1968 and 1972 and the long term mean. The figure suggests that August 1964 was indeed wetter than the mean in the Sahel region but drier in the region between 5°N and 10°N. The curve for August 1968 further illustrates the relatively limited northward migration of the zone of maximum precipitation inferred from Fig. 2. August 1972 seems to have had decreased rainfall for the whole region illustrated, with a centre of maximum precipitation slightly south of that of the mean.

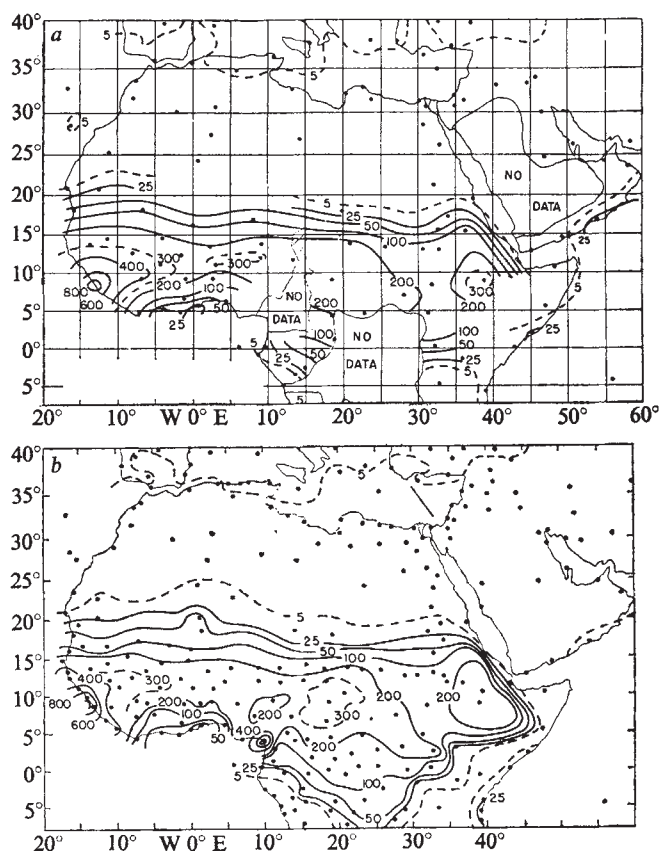


Fig. 1 Analyses of average August precipitation data (mm) for 1941–50 (a) and 1951–60 (b) for the African region. Dots locate positions of stations which usually report in the respective decade.

Figure 4 portrays five possible measures of the 'intensity of drought' for the Sahel. The lower two curves show the arithmetic mean August and annual precipitation of eight stations along 14°N. The stations were picked because of their proximity to the chosen latitude, their relatively equal amounts of August and annual rainfall, and their nearly continuous records since 1941. El Fasher was chosen as the most easterly station considered because data from sites near the Nile basin and Ethiopian mountains tend to obscure the clear trends in the region west of about 30°E (Figs 1 and 2). These curves show that the patterns in time of August and annual data are very similar. The wettest five-year period was that from 1950 to 1954, which had about 120% of the long term annual mean. The driest was 1968–72, with about 80% of that mean.

The middle curve of Fig. 4 shows a line drawing of the average August precipitation along 15°N between 10°W and 25°E obtained from the entire set of analysed maps. The curve directly above indicates the average latitude of the 100 mm isohyet in the Sahel between 10°W and 25°E as read from the same maps. The lines for the 1951–60 means are the

Fig. 2 Analyses of August precipitation data for 1964 (a), 1968 (b), and 1972 (c) for the African region.

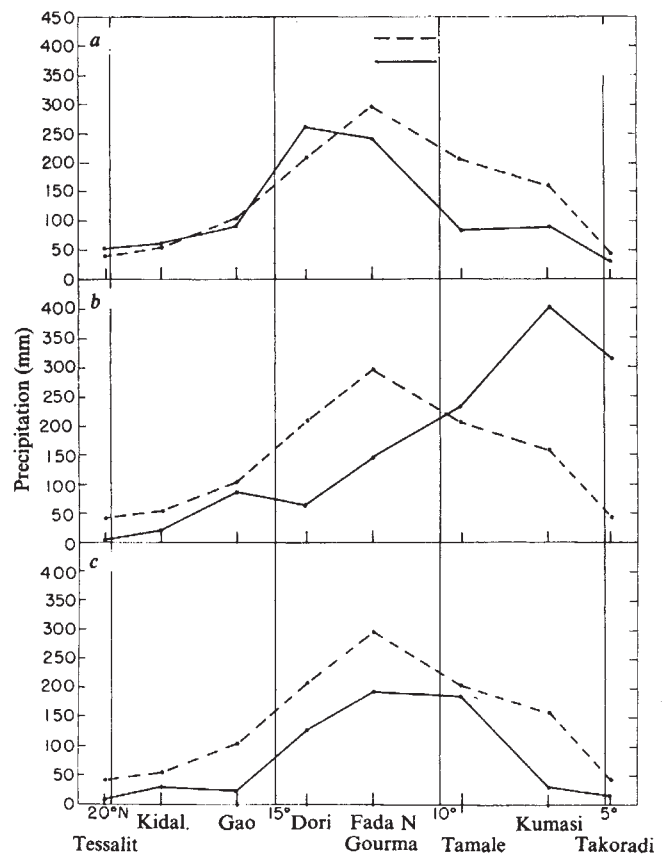
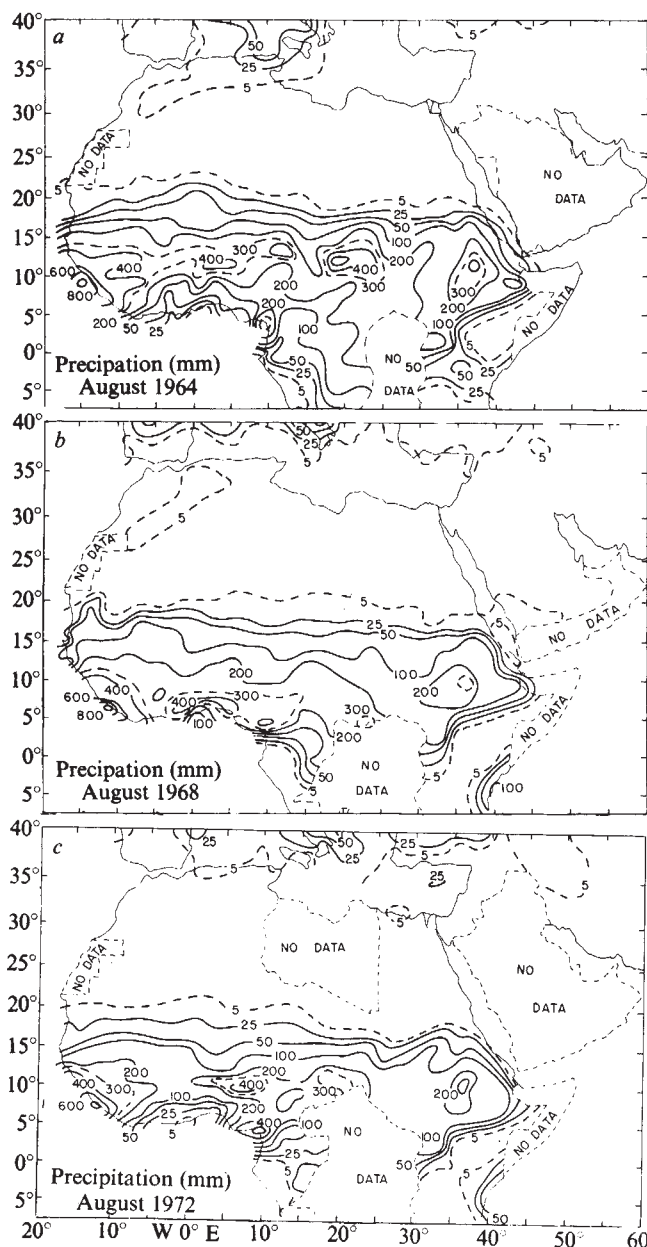


Fig. 3 Plots of August precipitation data for the eight stations listed, which are approximately along the prime meridian, for 1964 (a), 1968 (b), and 1972 (c), together with the long term mean. Station positions are: Tessalit, 20°12'N, 0°59'E; Kidal, 18°26'N, 1°32'E; Gao, 16°16'N, 0°37'W; Dori, 14°2'N, 0°3'E; Fada N Gourma, 12°04'N, 0°21'E; Tamale, 9°25'N, 0°53'W; Kumasi, 6°43'N, 1°37'W; Takoradi, 4°53'N, 1°46'W. —, Climatology; —, precipitation.

result of similar readings of the 1951–60 map (Fig. 1). The top curve of Fig. 4 indicates the approximate latitude of the centre of maximum rainfall (centre of gravity) near the prime meridian as interpolated from the August analysed maps. Figure 4 clearly shows that 1968–73 was the driest six-year spell of the period covered by good records.

The slight discrepancies between the five curves further indicate the difficulty in determining a single quantitative measure of regional precipitation patterns.

The 12-month running means of the entire record of several stations having data for the years before 1941 (including Accra, 5°33'N, 0°12'W; El Fasher, 13°38'N, 25°20'E; Kano, 12°3'N, 8°32'E; Khartoum, 15°36'N, 32°33'E; Lungi, 8°37'N, 13°12'W) were computed. Taken as a whole, these tend to confirm Jenkinson's⁴ conclusion that there was a severe drought for a three-year period centred on 1913 and a less severe one around 1926. In addition September volume flow measurements⁶ of the Niger River at Kayes indicate that the flow in 1913 was about 28% of the mean for 1903–68, the lowest reported up to 1968. The flow in 1926 was 59% of the mean. The running means were also compared to the curves of Fig. 4. Unfortunately, each station failed badly to correlate with the curves in at least one distinct year with the exception of Kano, which has an unfortunate lack of data in the critical period after 1966. This finding, combined with the fact that there are only a few poorly distributed stations in the Sahel for the earlier period, seems to preclude a comparison of the severity of earlier droughts with the most recent for the broad Sahelian region.

Spatially, both the position of maximum rainfall and the northward gradient from that position are necessary to specify the precipitation pattern over the Sahel. In addition, the

Table 1 Climatic data from analysed global maps

	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973
Global average 700-mbar height at 50°N in January	2,850	2,879	2,879	2,864	2,857	2,838	2,859	2,870	2,870	2,840	2,867	2,837	2,872
Global average 700-mbar height at 50°N in August	3,064	3,053	3,061	3,041	3,044	3,057	3,054	3,045	3,053	3,058	3,052	3,065	3,069
700-mbar height over the Sahara at 25°N in August	3,215	3,220	3,205	3,215	3,208	3,220	3,215	3,240	3,240	3,230	3,220	3,215	3,230
700-mbar height over the Atlantic at 25°N in August	3,209	3,200	3,210	3,210	3,215	3,210	3,220	3,210	3,210	3,215	3,210	3,220	3,210
Latitude of 700-mbar high over the Sahara in August	30°N	32°N	30°N	29°N	30°N	27°N	30°N	27°N	25°N	30°N	30°N	25°N	30°N
Latitude of 700-mbar high over the Atlantic in August	32°N	33°N	33°N	33°N	30°N	32°N	32°N	27°N	32°N	32°N	33°N	32°N	32°N

patterns of precipitation for the large area of plain west of about 25°E are only related to those east of that longitude in a complex fashion. This is perhaps not surprising if one considers the orography of the eastern region and the difference in average wind patterns of that region compared to those of the central and western Sahel¹. So Khartoum and other Sudan stations are not representative of the large scale trends and are therefore probably of little value in estimating trends before 1941. Finally, the 1968–73 period was indeed the most distinctive since 1941, although the period around 1913 may be comparable. Preliminary reports suggest that Sahelian rainfall was near normal in 1974. The eight-station average for August 1974 is indicated in Fig. 4.

Winstanley⁶ has suggested that Sahelian rainfall is negatively correlated with the southernmost position of the troughs in the mid-tropospheric circumpolar westerlies as inferred from winter-season 500-mbar analyses at 40°N between 110°W and 70°E. His results have been interpreted as a migration of

the Sahara southwards during 1968–73. We have prepared 700-mbar height analyses for January and August for the years 1961–73. From these, values were read at each 10° meridian around 50°N. The averages of these heights are given in Table 1. Given the relative randomness of the results for January and the large standard deviations within a latitude circle (about 95 geopotential metres, gpm) there does not seem to be any trend in these heights which could be correlated with Sahel rainfall trends (see also ref. 7).

In Table 1 we also present estimates of the latitude of the August subtropical high and the 700-mbar height at 25°N in the regions of the Sahara and the central Atlantic. Neither the Atlantic data nor the August global averages of 700 mbar height at 50°N (standard deviations about 30 gpm) show a clear trend. The Atlantic estimates would not support Bryson's⁸ contention that Sahelian rainfall patterns can be inferred from the position of the subtropical high over the Atlantic. Namias⁹, on the other hand, has reported evidence of negative correlations between the geopotential over northeast Europe and the rainfall for three stations in the Sahel for the summer season. Both the latitude of the subtropical high and its height at 25°N north of the Sahel suggest a southerly migration of that high by about 2° of latitude from 1961–65 to 1969–73. The cumulative regional and global geopotential evidence suggests a slight change in the morphology of the subtropical high pressure belt and the trough of the mid-latitude westerlies during 1968–73, but does not seem to be consistent with a suggestion of a global expansion southward of either.

Available German Weather Service global surface temperature departure maps¹⁰ have been supplemented by our own plots of the station data to provide analyses for January, July and August of 1961–73. The August temperatures of the Sahel were warmer during 1968–73 than the long term mean, with departures exceeding 2 °C in 1968 and 1972. Satellite measurements suggest that this can be attributed, in part, to increased solar heating at the surface due to the decrease in cloudiness and consequent change in overall albedo during this relatively dry epoch. In 1969, for example, the total albedo over the Sahel was about 35% in July and 26% in the following dry month of October¹¹.

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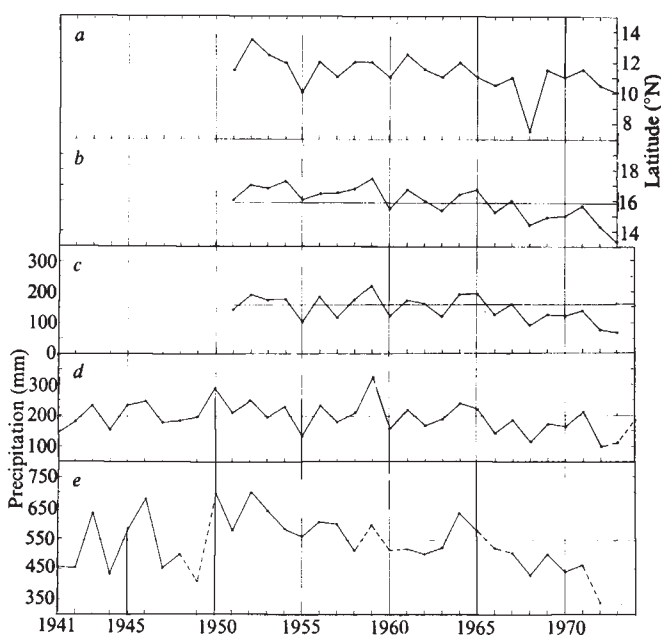


Fig. 4 Plots of five measures of rainfall for the Sahel region from 1941 or 1951 to 1973. The data of the upper three curves were read from analysed August precipitation maps. The bottom two curves indicate the averages of the station reports at: Tambaconda, 13°46'N, 13°41'W; Kayes, 14°26'N, 11°26'W; Mopti Severe, 14°31'N, 4°6'W; Gao, 16°16'N, 0°37'E; Niamey, 13°39'N, 2°10'E; Zinder, 13°48'N, 9°0'E; Abecher, 13°51'N, 20°51'E; El Fasher, 13°38'N, 25°20'E. Dashed lines indicate one station report has been objectively interpolated using the long term means and that year's actual reportings for the other seven stations. *a*, Latitude of centre of gravity, 5°W–5°E; *b*, August latitude of 100 mm precipitation, 10°W–25°E (solid line, 1951–60 mean); *c*, August precipitation along 15°N, 10°W–25°E (solid line, 1951–60 mean); *d*, eight-station mean August precipitation (solid line, 1941–70 mean); *e*, eight-station mean annual precipitation (solid line, 1941–70 mean).

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letters to nature

Magnetic field of Mercury confirmed

ON March 16, 1975, the US spacecraft Mariner 10 underwent its third and final encounter with Mercury. This unique and fortuitous event occurred because the heliocentric orbital period of Mariner 10 was 176 d, exactly twice that of the orbital period of Mercury. The principal objective of the third fly-by was to confirm or reject the suggestion from the first fly-by on March 29, 1974 that Mercury may possess a modest intrinsic magnetic field sufficient to deflect the solar wind flow^{1,2}. The quantitative analysis³ of the Mercury I data yielded an estimate of the planetary magnetic dipole moment equal to 5.1×10^{22} gauss cm^3 and oriented 7° from the orbit normal. The spacecraft had not been functioning properly since Mercury I because of various technical failures, but the spacecraft engineering team at the NASA Jet Propulsion Laboratory (JPL) managed to achieve the desired objective at Mercury III of a very close dark-side pass. Here we present preliminary results from the NASA Goddard Space Flight Center magnetometer instrumentation on Mariner 10.

The fly-by trajectory is shown in planet centred solar ecliptic coordinates in Fig. 1. This is a preliminary trajectory and will eventually be updated by means of tracking observations made after the encounter. The nominal 'miss distance' at closest approach was approximately 323 ± 8 km. For comparison, also shown in Fig. 1 is the trajectory at Mercury I encounter, for which the miss distance at closest approach was 704 km. In Fig. 1b, showing the view from the Sun, the much higher latitude of the spacecraft pass during Mercury III is well illustrated. This closer and more poleward targeting was chosen so as to provide magnetic field measurements which would provide unequivocal evidence about the nature of Mercury's magnetic field. The Mercury I results were consistent with a reduced-scale model of the interaction of the solar wind with the terrestrial magnetic field, in which the planetary field is compressed by the solar-wind flow (itself deflected around the planet) and a detached bow shock wave forms. As a result, the field is

then confined to a region close to the planet on the Sunward side and extended to form a magnetic tail on the night side.

Based on the observations and analysis at Mercury I and assuming similar conditions of solar-wind momentum flux at Mercury III, predictions were made of the locations of the characteristic bow shock and magnetopause boundaries (Fig. 1a). Since the rotation period of the planet is uniquely coupled to its orbital period, in the ratio 2:3, the 'phase' of the planet relative to the planet-Sun line at successive encounters was identical.

The observed positions of the bow shock and magnetopause during Mercury III are also illustrated in Fig. 1. They show excellent agreement with the model boundaries, which used a scale magnetic moment of Mercury equal to 7×10^{22} that of the Earth's dipole moment. In the case of the solar-wind flux impinging normal to a dipole field, a theoretical study⁴ has yielded the relationship

$$R_{\text{mp}}/R_p = 1.07 [B_0^2/4\pi mnV^2]^{1/6}$$

where R_{mp}/R_p is the normalised radius of the stagnation point distance of the magnetopause, B_0 the equatorial dipole field intensity, and mnV^2 the solar-wind momentum flux (where n is the number density, m the mass of the proton and V the velocity of the solar wind). The distance to the magnetopause, as measured by R_{mp} , thus depends weakly on the solar-wind momentum flux and, therefore even if variations of $\pm 60\%$ in the solar-wind flux occur, they would alter the position of the characteristic bow shock and magnetopause boundaries by only $\pm 10\%$.

The magnetic field measurements were accomplished with a dual magnetometer system⁵ which sampled the vector magnetic field 25 times a second, with a precision of $\pm 0.125 \gamma$ for each orthogonal component. These data are to be considered preliminary in that they are accurate to $\pm 1-2\gamma$ or $\pm 1\%$, whichever is the larger, because they are derived from quick-look data provided in near real time by the JPL. Six-second averages of the observed magnetic field throughout the encounter period are

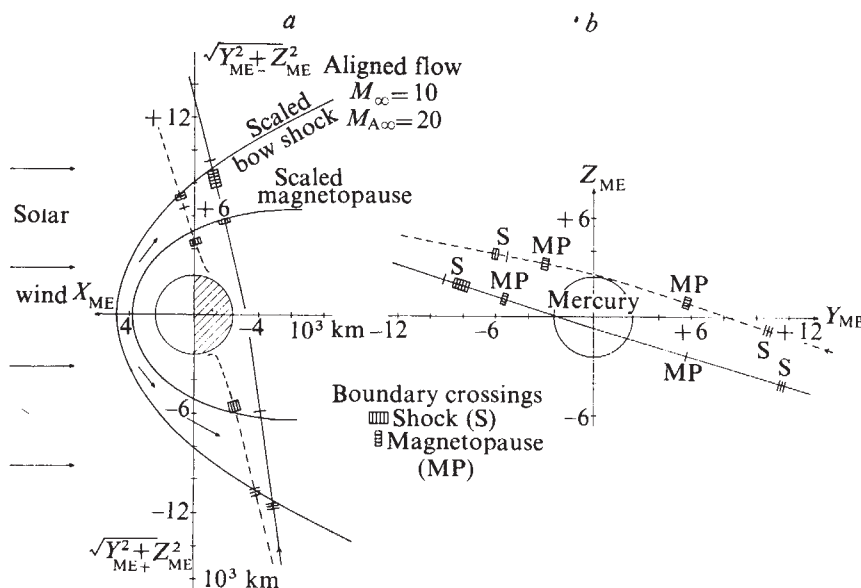


Fig. 1 Mariner 10 spacecraft trajectory in Mercury-centred solar ecliptic coordinates. a, Trajectory plotted as distance, X_{ME} , from the dawn-dusk terminator of the planet against distance from planet-Sun line, $(Y_{\text{ME}}^2 + Z_{\text{ME}}^2)^{1/2}$. View from the Sun with Z_{ME} positive to north ecliptic pole. ---, Mercury III; —, Mercury I.

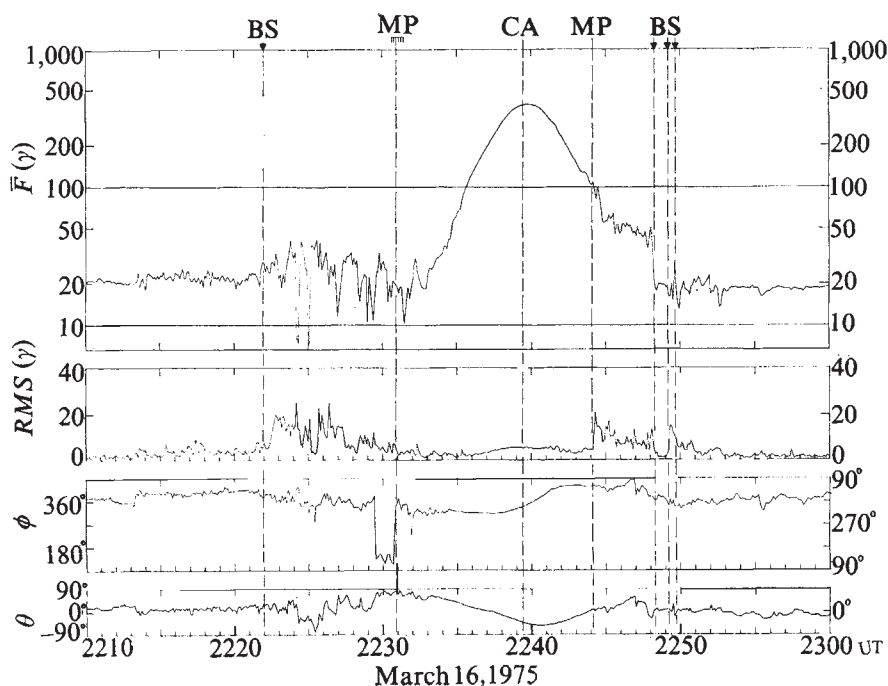


Fig. 2 Measurements by the NASA GSFC magnetometer on Mariner 10 during Mercury III. Magnetic field direction latitude, θ , is referenced with respect to a plane parallel to the ecliptic and longitude, ϕ , with respect to the planet-Sun line reckoned positive relative towards the east limb of the Sun. BS, Bow shock; MP, magnetopause; CA, closest approach.

shown in Fig. 2. The occurrence of the well developed bow shock and magnetopause boundaries are indicated and readily identified by a magnitude and/or directional change as well as in the Pythagorean mean fluctuation characteristic, RMS. The maximum magnitude of the field is 400γ , four times larger than that observed at Mercury I encounter and 20 times larger than the interplanetary field, which is close to 20γ . This large value precludes any reasonable possibility that the magnetic barrier to solar-wind flow is associated with a complex induction process occurring at the planet. The direction of the magnetic field, as measured by latitude θ and longitude ϕ , is primarily towards the Sun (and planet), with gradual changes along the trajectory within the magnetosphere boundaries. This orientation is

Table 1 Comparison of planetary dipole moments obtained by spherical harmonic analysis of magnetic field data

	Mercury I March 29, 1975	Mercury III March 16, 1975
Dipole moment (gauss cm^3)	5.1×10^{22} ($350 \gamma R_m^3$)	$4.8 (\pm 0.5) \times 10^{22}$ ($330 \pm 30 \gamma R_m^3$)
Latitude (ME coordinates)	-80°	$-76^\circ \pm 5^\circ$
Longitude (ME coordinates)	285°	$90 \pm 30^\circ$
Root mean square residual	0.9γ	7γ
Comment	Quiet data only	Preliminary but entire data set

opposite to that observed at Mercury I but is consistent with the model of a Mercurian magnetosphere (Y.C.W. and N.F.N., unpublished) in which the planetary field is represented by a centred dipole normal to the flow of the solar wind and which is then highly distorted by magnetopause currents.

A preliminary spherical harmonic analysis of the data within the magnetosphere has been carried out using the available trajectory. The analysis assumes a centred planetary dipole field (harmonic order $N = 1$) as well as external field sources described by harmonic terms up to order $N = 2$. The preliminary least-squares fit yields a residual of 7γ with the deduced parameters for the planetary dipole listed in Table 1.

These are compared with those values derived from Mercury I and are seen to be in remarkably good agreement, considering

the preliminary nature of these results. The magnitude of the dipole moment is the same within 10% and its direction only 24° from that derived earlier. The uncertainty associated with the present analysis means that we cannot attribute any significance to these small differences.

In summary, the observations at the third encounter with Mercury by the NASA GSFC magnetic field experiment have confirmed the earlier tentative conclusion that Mercury has a modest, intrinsic magnetic field, the origin of which is at present uncertain. It may be associated with remanent magnetisation of parts of the planet (material at present below its Curie point) or it may be caused by an active dynamo in the presumably large iron-nickel core. It is, however, difficult to construct a plausible sequence of events leading to the model in which the Mercurian magnetic field is explained in terms of remanent magnetisation, so we conclude tentatively that these observations require the existence of an active dynamo mechanism.

We thank Dr James Dunne, JPL Project Scientist on Mariner 10, and the JPL spacecraft team for their outstanding effort in achieving the third encounter. We also appreciate the special efforts of D. H. Howell, F. W. Ottens and R. F. Thompson of the NASA GSFC for their special efforts and prompt analysis of these data.

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Preliminary interpretation of plasma electron observations at the third encounter of Mariner 10 with Mercury

OBSERVATIONS of electrons with energies between 13.4 and 690 eV made at the first encounter of Mariner 10 with Mercury (Mercury I) unexpectedly showed¹ the presence of a strong bow shock wave, enclosing a magnetosheath and magnetosphere with boundaries the positions of which could be explained by assuming Mercury to possess an appreciable magnetic moment, in agreement with, and complementary to, the observations made by the magnetometer carried by the spacecraft². Inside these boundaries were found distinct distributions of magnetospheric particles.

A second encounter took place on September 21, 1974, but the spacecraft passed the planet on the Sunward side at too great a distance to permit observations inside the planet's bow shock. The third, and last, encounter (Mercury III) took place on March 16, 1975 at which time the spacecraft passed within 330 km of the surface of the planet, along the trajectory shown in Fig. 1. The primary purpose of this close polar approach was to determine whether the magnetic moment of the planet was intrinsic and to enable further observations to be made inside the magnetosheath and magnetosphere.

The results presented here are from an electron spectrometer mounted on a scan platform. It accepts electrons from the anti-solar direction in the energy range 13.4–690 eV in 15 logarithmically spaced channels of width $\Delta E/E = 6.6\%$. The instrument was mechanically scanned at a rate of 1° per second through an arc of 120° centred on a direction in the ecliptic plane 6° east of the spacecraft–Sun line. The field of view is fan shaped, $\pm 13.5^\circ$ in a plane containing the scan axis and $\pm 3.5^\circ$ in a plane perpendicular to the scan axis, which is maintained in the direction of Canopus by the attitude-control system of the spacecraft. The instrument obtains an electron spectrum every 6 s, from which the density, temperature and so on can be obtained¹.

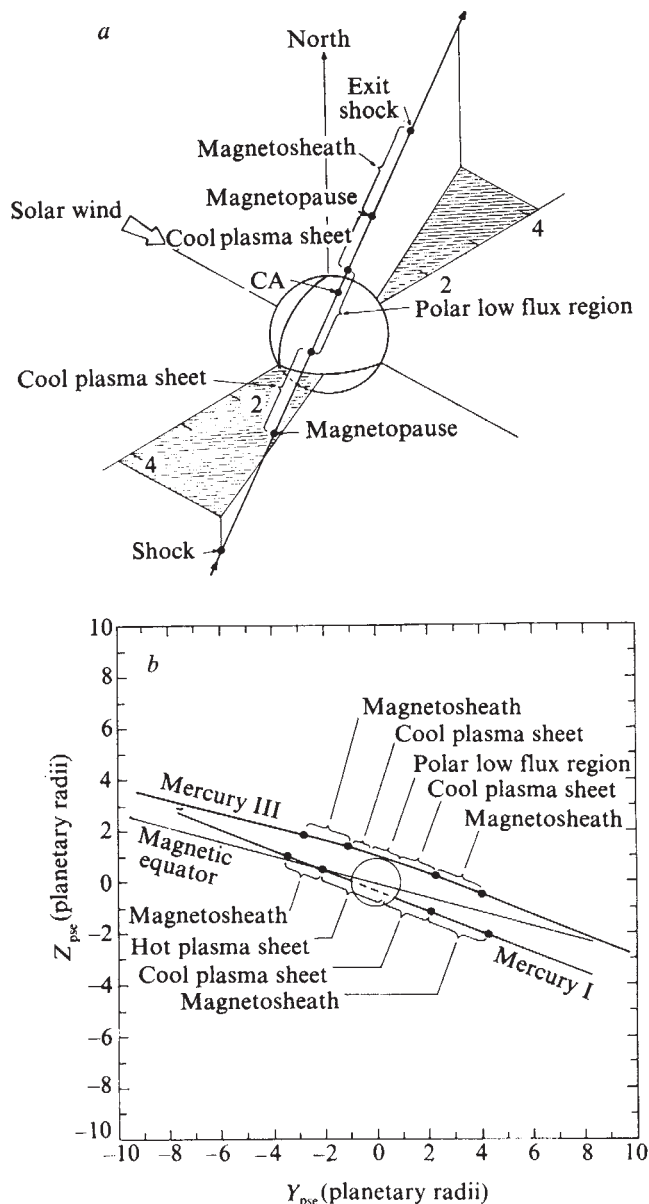
Figure 1a shows an isometric view of the Mercury III trajectory and Fig. 1b a view from the Sun of the trajectories at both Mercury I and III, with the locations of observed plasma features marked. Entering and leaving the interaction region at each encounter, the plasma instrument detected the bow shock, the magnetospheric boundary and the intervening shocked solar-wind plasma (the magnetosheath). Within the magnetosphere, distinctive plasma electron populations were observed which exhibited large spatial and/or temporal variations. The magnetospheric populations observed at the two encounters differed significantly (Fig. 1b). Figure 2 shows electron fluxes in two energy channels for both encounters. Features labelled in Fig. 1 are identified here with vertical lines. The plasma signatures for the bow shock, magnetosheath and magnetospheric boundary seem to be similar in character at both encounters¹. In the magnetosphere the Mercury I data show a transition between a cooler and a warmer electron population on the inbound and outbound trajectories. The Mercury III magnetospheric data show a sequence beginning with a cool plasma followed by a region of low fluxes in all energy channels and then returning to a cool electron population.

Spectra of the electrons from different plasma populations observed during Mercury III are shown in Fig. 3a, and a comparison with the "hot plasma sheet" spectra from Mercury I is made in Fig. 3b. The solar-wind spectrum exhibits the characteristic two-temperature profile. The expected increase in flux at all energies behind the bow shock is evident in the magnetosheath spectrum. The spectra of the "cool plasma sheet" populations measured at the two encounters are similar at energies above approximately 50 eV, but are quite different at lower energies; this difference is tentatively attributed to

spacecraft charging. The plasma sheet is characterised by a flux above 100 eV which has a temperature even higher than that of the halo electrons in the solar wind, whereas the electrons in the low flux region have a temperature lower than those in the solar wind.

The magnetopause and bow shock were found in Mercury I to be geometrically similar to those of the Earth but reduced in size relative to the planet by a factor ~ 6 . The present observations support this interpretation and provide further details of the plasma electron populations in Mercury's magnetosphere. Scaled up to the size of the Earth's magnetosphere, the Mercury I trajectory corresponds to a crossing of the Earth's magnetospheric tail from solar magnetospheric coordinates $X_{SM} \approx -10R_e$ (Earth radii) and $Z_{SM} \approx -3R_e$ on the dusk magnetopause to $X_{SM} \approx -5R_e$ and $Z_{SM} \approx 0R_e$ on the dawn magnetopause. The trajectory of Mercury III corresponds to a

Fig. 1 *a*, Isometric drawing of the trajectory of Mariner 10 during its third encounter with Mercury. Features of the interaction described in the text are shown along the trajectory. CA, Point of closest approach (327 km, at 2248:30 Earth-received time on March 16, 1975). *b*, View from the Sun of Mercury I and Mercury III, showing the approximate magnetic equator and the regions sampled during each encounter. The coordinate system is solar-ecliptic.



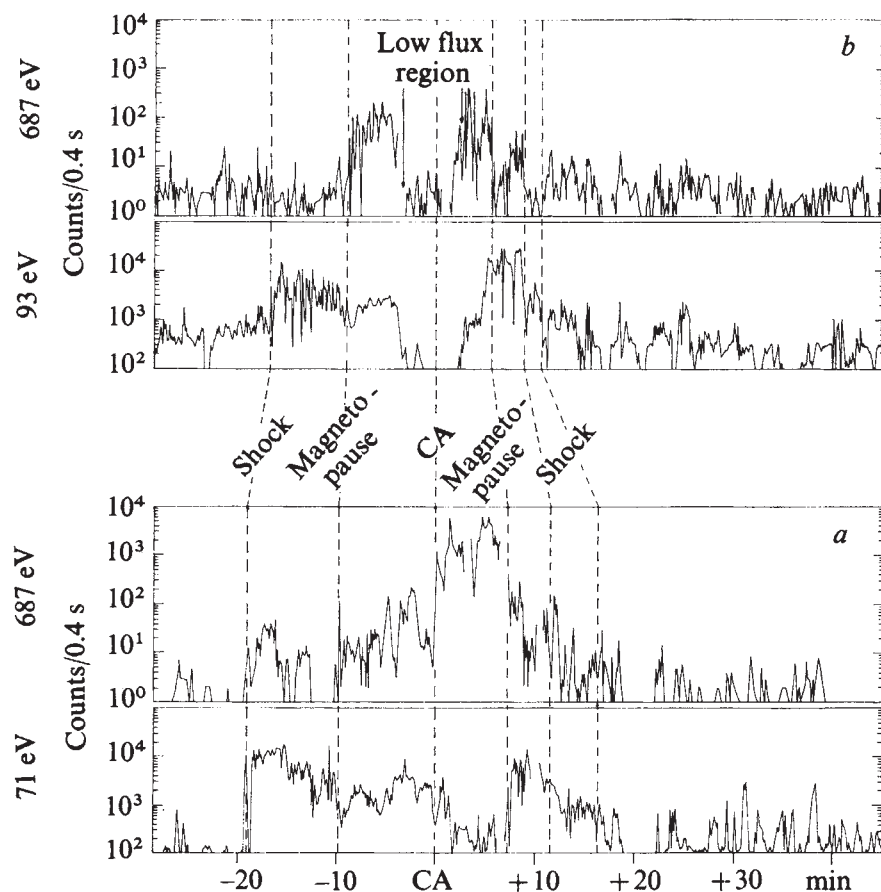


Fig. 2 Electron fluxes measured at 71 eV and 687 eV during Mercury I (a) and at 93 eV and 687 eV during Mercury III (b), with the bow shock, magnetopause and low flux region indicated by vertical lines. The curves have been aligned so that times of closest approach coincide.

similar crossing from $X_{SM} \approx -6R_e$ and $Z_{SM} \approx +4.5R_e$ to $X_{SM} \approx 0R_e$ and $Z_{SM} \approx +6R_e$. (The X_{SM} axis is the planet-Sun line. The Z_{SM} axis is positive northwards, defined such that the X_{SM} - Z_{SM} plane contains the dipole axis. These approximate solar magnetospheric coordinates are based on the Hermean magnetic dipole deduced from Mercury I magnetic field observations².) Both the present plasma observations and, in retrospect, those from Mercury I indicate large scale properties of the electron populations very similar to those which would be observed along scaled-up trajectories through the Earth's magnetosphere. The Earth's magnetospheric tail contains a plasma sheet centred about the solar magnetospheric (SM) equatorial plane and extending to a thickness of several R_e , characterised by a hot particle population (mean electron energy ≈ 1 keV) near the centre and a cool population ($\approx$

100–200 eV) near the edges; above and below the plasma sheet, on magnetic field lines connected to the polar caps, the particle intensities are very low. Figure 2 shows that all these regions have now been sampled at Mercury, at roughly the locations expected on the basis of scaling from Earth observations. The cool electron population observed at a relatively large distance from the SM equatorial plane by Mercury III is characteristic of the outer plasma sheet near the midnight meridian and at its largest distance from the SM equator, a region of low particle intensities which seems to be the analogue of the Earth's polar cap. At Mercury I, the spacecraft entered the magnetosphere at some distance from the SM equator and also observed a cool electron population, but on its outbound trajectory it was near the SM equator and observed a hot electron population similar to that in the central region of the Earth's plasma

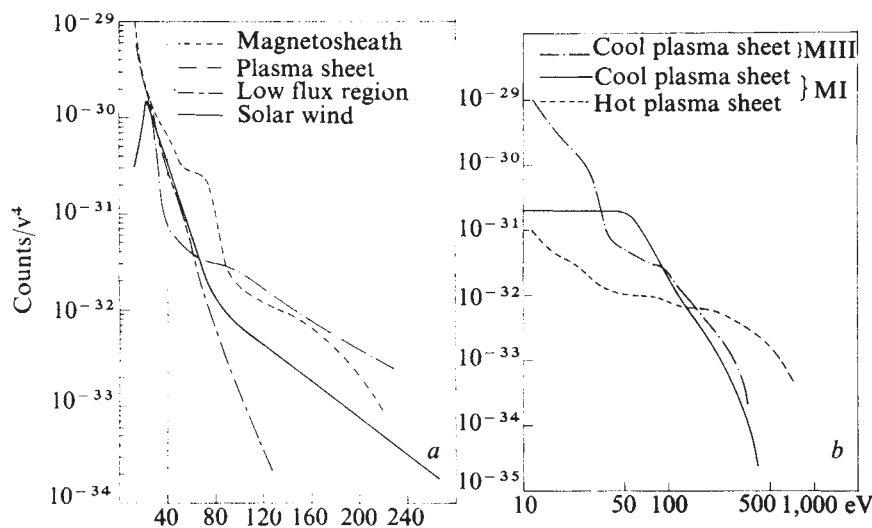


Fig. 3 a, Spectra of electrons observed at Mercury III. The vertical dotted line at 40 eV indicates distortion of the spectra below the energy as a result of spacecraft charging. The ordinate (counts/ v^4) is proportional to the distribution function. b, Comparison between cool and hot plasma sheet spectra from the Mercury I observations with a cool plasma sheet spectrum from Mercury III.

sheet; as expected from its trajectory, it was never far enough from the SM equator to reach the low flux polar cap region.

The characteristic electron energy in the cool plasma sheet (Fig. 3b) observed in both Mercury I and III is ~ 100 –200 eV, whereas in the hot plasma sheet observed in Mercury I it is at least ~ 1 keV; these energies are of the same order of magnitude as those observed in the corresponding regions of the Earth's plasma sheet. The fact that the plasma sheet energies in both magnetospheres are comparable implies that the plasma sheet energy does not scale with the electric potential difference across the magnetospheric tail, the value of which for Mercury should be about one-seventh of that for the Earth³; this has important implications for the still unsolved problem of the origin of the plasma sheet.

In summary, well defined bow shock and magnetospheric boundary crossings were observed on the inbound and outbound trajectories during Mercury I and III. The locations of the boundaries observed are mutually consistent with locations obtained from models which scale the Earth's magnetosphere to Mercury I data^{1,4} and the other magnetospheric features are consistent with the magnetic field model⁴. Thus, the plasma results strongly support the idea that Mercury's magnetic field is intrinsic rather than induced, and show that the magnetosphere of Mercury is remarkably similar to that of the Earth.

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Possible identification of Ariel 1118–61

THE transient X-ray source Ariel 1118–61 has been discovered by Ariel V (refs 1, 2) and the best estimate for the position is (1950.0) $\alpha = 11$ h 18 min 59s, $\delta = -61^\circ 35.3'$. We point out here the presence in the error box of the long period Mira-type variable RS Cen (1950.0) $\alpha = 11$ h 18 min 16.5 s, $\delta = -61^\circ 36.0'$. It has been suggested that in the Mira-type variables α Ceti (Mira) and SY For, the blue variable companion is a white dwarf illuminated by infalling material accreted from the strong wind of the M-type star^{3–5}. The similarity between these systems and the (recurrent) nova T CrB has also been stressed⁵. We propose that some transient X-ray sources are binary systems consisting of a compact object in orbit around a long period variable. The accretion rate of material on to the compact object in such a system⁴ is $\lesssim 10^{-8} M_{\odot} \text{yr}^{-1}$ which is just the rate required to produce X rays from a compact object. We note, too, the proposed identification⁶ of another transient X-ray source (2U1543–47) with a variable late-type giant.

The large variability of radius and luminosity of a Mira-type star is likely to produce strong modulation of the stellar wind and a similar modulation of the accretion rate on to and thus

luminosity of the compact object. A similar correlation between stellar wind strength and luminosity of the X-ray object has been proposed for Cen X-3 (refs 7, 8). We expect the modulation to be much stronger for Mira-type stars.

The transient source A1118–61 was observed to peak in mid-December 1974. The period of RS Cen is⁹ 164.51 d and the X-ray source appeared at phase ~ 0.4 (optical maxima occur at phase 0.0 and minima at 0.54) of the cycle when the radius of RS Cen is expected to reach a maximum. We intuitively expect mass outflow to be greatest near light minimum, when absorption bands are strongest in the stellar atmosphere. If the proposed identification is correct we expect the transient X-ray source to appear 'regularly' with a period of 164 d. Eclipses of the compact object by the red variable may complicate this prediction: the orbital period for such a system is $\gtrsim 2$ yr. We would associate the 6.75 min periodicity of the X-ray object with the rotation (or possibly precession) period of the compact object¹⁰.

The identification of A1118–61 with RS Cen would establish the identity of a new class of X-ray binaries. Such systems would offer a unique opportunity to study the structure and dynamics of the atmospheres and winds of Mira-type variables.

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Observations of low energy gamma-ray bursts with SAS-2

Low energy celestial gamma ray bursts were discovered¹ in 1973 and the detection later confirmed by several groups. In this paper we report the low energy gamma-ray bursts observed by the plastic scintillator anticoincidence dome of the Small Astronomy Satellite-2 (SAS-2) gamma-ray telescope. Although the high energy gamma-ray telescope of SAS-2 has a threshold of about 20 MeV, the large SAS-2 anticoincidence dome (A-dome), which is the outermost element of the instrument, provides a very large detector for the high energy portion (≥ 0.3 MeV) of these burst events.

Details of the instrument are given by Derdeyn *et al.*². The A-dome, constructed out of 1.5 cm thick scintillator has an average collection area of about $2.5 \times 10^3 \text{ cm}^2$ and is an almost omnidirectional detector, only being insensitive to directions within a small cone ($\sim 30^\circ$ half angle) centred around the direction that it is attached to the spacecraft. The average effective threshold for detection over the dome is about 0.15 MeV, the average efficiency rising to about 20% at 0.6 MeV. The counting rate of the A-dome is accumulated and read out every 768 ms. When there is no increase resulting from the trapped radiation in the Atlantic anomaly, the observed rate is about $4.2 \times 10^3 \text{ counts s}^{-1}$ and it remains quite steady. (The Earth's trapped radiation extends downward to unusually low altitudes in the central

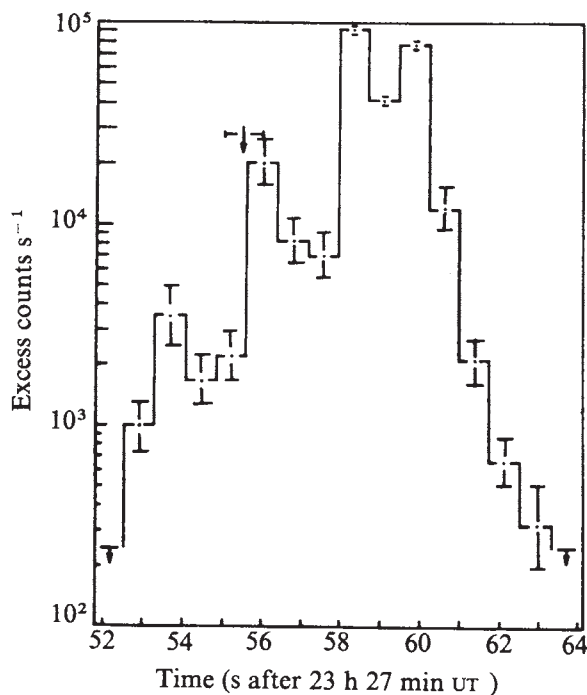


Fig. 1 Time history of the low energy gamma-ray burst observed in the SAS-2 A-dome on March 2, 1973. The arrow shows the event time observed by the Vela satellites. The counting rates shown are the excess counts above the steady SAS-2 background of 4.2×10^3 counts s^{-1} .

and south Atlantic. These low energy electrons and protons cause an increase in the count rate of the A-dome.)

SAS-2 was launched on November 15, 1972 into an equatorial orbit with a 440-km perigee and 610-km apogee. During the period of experimental operation (November 20, 1972–June 8, 1973, the counting rate from the A-dome provided a continuous monitor for fluctuations in the low energy gamma-ray fluxes from the unocculted sky. Using these data a search has been made for sudden rate increases where there is an excess of 600 counts or more over the background in at least three successive 0.768 s readouts. Although this criterion would not pick up events with time scales shorter than 2 s, the occasional noise spikes in single

readouts made its adoption necessary. The selection criterion is further biased against events which last longer than 30 s because of background comparisons which were made on this time scale.

This procedure resulted in the detection of two events observed by other satellites and the discovery of one, which has now been confirmed by other satellite observations. In addition, two events on the list of those detected by the Vela satellite series³ were not seen by SAS-2. In view of the high sensitivity of SAS-2, these negative results suggest that the Earth occulted the source, as it will, on average, 40% of the time. Table 1 lists the five events that have been confirmed as multisatellite observations. There was no reported solar activity during any of these events.

Considering the two events not seen by SAS-2, if it is assumed that the absence of a detectable signal in the SAS-2 A-dome is an Earth occultation effect rather than a large variation in the expected energy distribution, then the regions of the sky in which the events occurred may be restricted regions. These are a 70° cone about $\alpha=36.6^\circ$, $\delta=1.0^\circ$ in the case of the December 18, 1972, event and a similar cone about $\alpha=27.4^\circ$, $\delta=1.6^\circ$ in the case of the May 7, 1973, event. For the December 18, 1972, event this cone is consistent with the location of the source lying in the region $70^\circ < \alpha < 120^\circ$, $0^\circ < \delta < 20^\circ$ as determined by Imhof *et al.*⁴ except that it excludes the highest part of the α range. The time history of the March 2, 1973, event as measured by SAS-2 is shown in Fig. 1. Notice that the time given by the Vela satellites for the first detection of an increase is well into the event. These data support the point of view that the gamma-ray bursts observed have a complex time structure and may have durations in excess of those measured by Vela type detectors that trigger on sudden increases⁴.

Even with the SAS-2 time resolution, four distinct peaks appear indicating a significant temporal structure (Fig. 1). The event reaches a peak intensity of 9.4×10^4 counts s^{-1} , more than two orders of magnitude greater than the threshold of 7×10^2 counts s^{-1} which the event must surpass in the 0.768 s readout interval in order to be selected in the computer scan of the SAS-2 data. Considering the occultation of SAS-2 by the Earth, the event must have occurred in a region of the sky within a cone of 110° around $\alpha=49.4^\circ$, $\delta=0.1$. The Vela data (R. W. Klebesadel, R. A. Olsen and I. B. Strong, private communication) indicate the source was in a circular band of radius $82.5 \pm 4^\circ$ centred at $\alpha=193.9^\circ$, $\delta=25.7^\circ$.

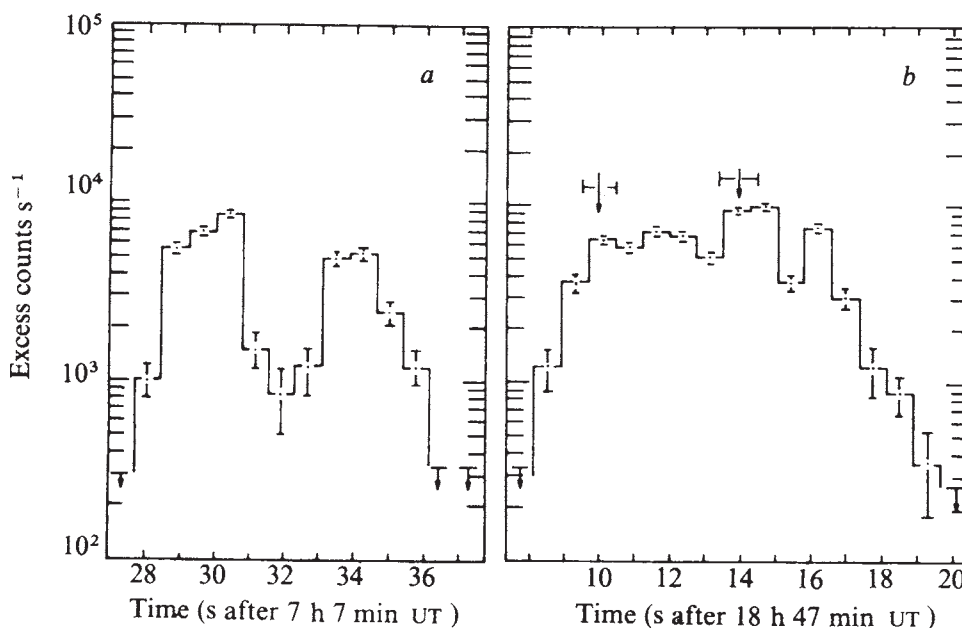


Fig. 2 Time histories of two gamma-ray burst events seen by the SAS-2 A-dome on June 6, 1973. The arrows show the event times observed by the Vela satellites. a, Vela 5A; b, Vela 6A. The counting rates shown are the excess counts above the steady SAS-2 background of 4.2×10^3 counts s^{-1} .

Two events seen by SAS-2 occurred on the same day, June 6, 1974, which is surprising considering the average event rate. The time profiles of these two events are shown in Fig. 2. There were only 12 detected cases in the approximately 6.5 months that the A-dome rate exceeded the threshold level and remained above background (by $>6 \times 10^2$ counts s^{-1}) for more than three readout intervals (approximately 2.3 s) except when the satellite passed through the Atlantic trapped radiation anomaly. The second June 6 event was subsequently confirmed by the IMP-7 satellite³ and the Vela system (Table 1). Notice that again for the June 6 event which did trigger the Vela system the Vela time for first detection occurs after the event has started and reached a level almost an order of magnitude above the SAS-2 threshold. Considering the occultation of SAS-2 by the Earth for the two June 6, 1973, events, the first must have occurred within 110° of $\alpha=184.1^\circ$, $\delta=1.2^\circ$ and the second within 110° of $\alpha=317.7^\circ$, $\delta=-1.9^\circ$. The other events observed by SAS-2, but not confirmed by other satellites, seem to be genuine

Table 1 Confirmed multisatellite observations (November 20, 1972–June 8, 1973)

Date	Time h min s	Satellite and reference
December 18, 1972	20 27 39	2 Vela*; IMP-7 ⁵ ; 1972-076B ⁴
March 2, 1973	23 27 53	2 Vela*; IMP-7 ⁵ ; SAS-2
May 7, 1973	08 04 32	3 Vela ²
June 6, 1973	07 07 28	IMP-7 ⁵ ; SAS-2
June 6, 1973	18 47 08	SAS-2; IMP-7 ⁵ ; 2 Vela*

The satellite listed first is the one from which data first led to recognition of the event.

*Unpublished results from R. W. Klebesadel *et al.*

events although the estimated fluxes are in the range of 3×10^{-6} to 10^{-5} erg cm^{-2} which may reduce the probability of detection by the other satellites involved.

In view of the significant sensitivity of SAS-2 as suggested by these comparisons and direction calculations, it may seem surprising that no other events with timescales between 2 and 30 s were observed; however, it is difficult to draw quantitative conclusions from this result because of the difficulty of comparing results from detectors with vastly different characteristics.

In none of the events was there any significant excess of high energy gamma rays in the spark chamber. This is not surprising since with the 0.25 sr solid angle of the high energy telescope, there is less than a 5% chance that an event will fall within the telescope acceptance angle even if there is a detectable flux of gamma rays with energies above 25 MeV.

We thank Drs Klebesadel, Strong and Olsen for providing unpublished Vela data and Drs Cline and Desai for reviewing the IMP-7 data to determine the existence of events at the times suggested by the SAS-2 data. H. Ö. is an NRC Senior Postdoctoral Associate.

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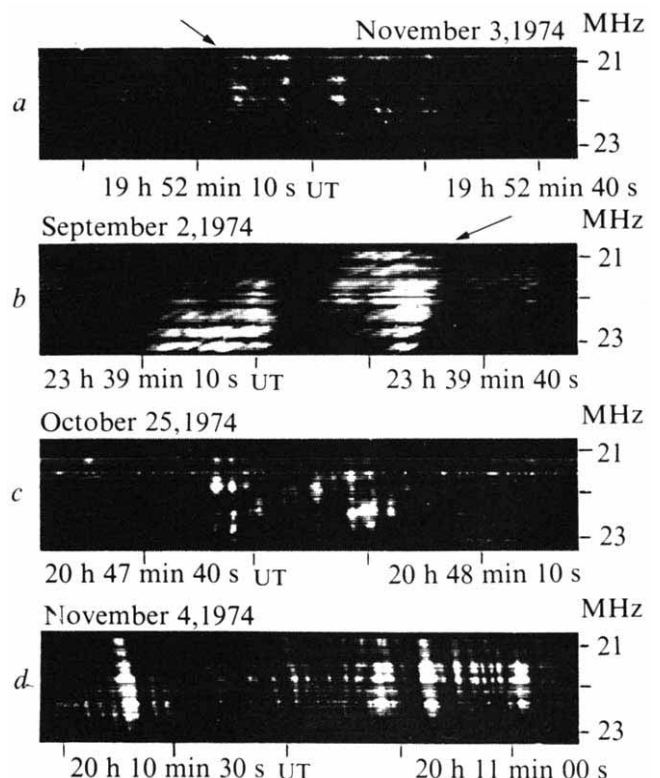
Polarisation structure of Jupiter's decametre radio bursts

The high-resolution dynamic spectra of Jupiter's decameter radio emission exhibit structures consisting of repeated, tilted ridges¹. The frequency–time slope of these 'diagonal patterns', also referred to as modulation lanes, varies from 50 to 300 kHz s^{-1} . The slope is a function of the System III central meridian longitude of Jupiter, and it may be either positive or negative^{2,3}. Recent observations of modulation lanes are described by Ellis^{4,5} and Lecacheux⁶. Here I report a study of polarisation diversity in the spectra of modulation lanes.

The frequency range 21–23 MHz is close to the region above which the Jovian bursts are predominantly elliptically polarised in the right hand (RH) sense, but below which the proportion of bursts polarised in the left hand (LH) sense increases. Since most of the spectra of the lanes have been recorded in RH circular or linear polarisation, no conclusive information on the relationship of the modulation lanes to polarisation is available⁷.

Rapid reversals of the sense of polarisation have previously been observed in spectral records of groups of S bursts⁸. The phenomenon occurred as a function of time and frequency, and was referred to as 'polarisation diversity'. This polarisation diversity has been observed^{9–12} in fixed frequency records of L-bursts. The reversals of polarisation sense were often cyclic with time. To study the function of diversity in modulation lanes, the spectra of the RH and LH circular components must be recorded separately, as in the experiment reported here.

Fig. 1 Samples of high-resolution spectra of Jupiter's decametre radio bursts. Modulation lanes are shown in *a* (positive drift) and *b* (negative drift) indicated by arrows. The durations of the envelopes are long in *b* but short in *c*. The envelopes seem to be tilted in *d*. The wide, parallel horizontal lanes are residual Faraday fringes. In *b* the antenna polarisation was linear and the Faraday fringes seem to be very strong.



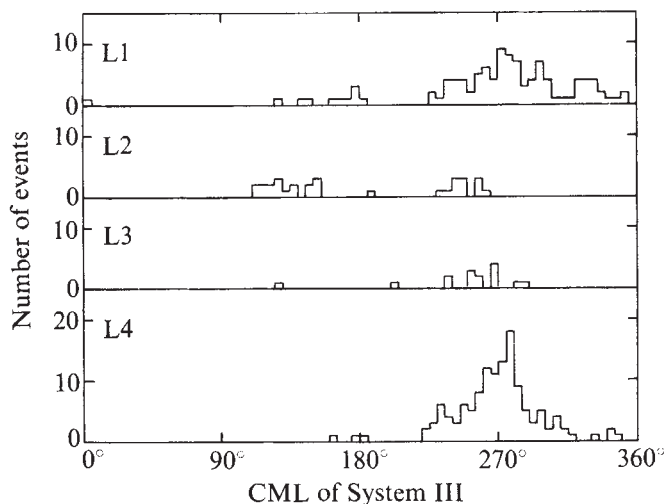


Fig. 2 Number of events of types L1 to L4 as a function of System III (1957.0) central meridian longitude (CML).

A new instrument was built for this purpose, the antenna of which is a steerable system of two cross log-periodic units in parallel. The circular components are obtainable with broadband quadrature hybrid circuits. The main equipment is a 48-channel receiver for the range 20.85–23.10 MHz. The channels are separated by intervals of 50 kHz and have 3 dB bandwidths of 15 kHz, with a signal integration time constant of 20 ms. The display unit consists of a two-colour (red and green) array of 96 light-emitting diodes, switched at a rate of 1 kHz in synchrony with the radio frequency switch at the output ports of the hybrid circuits.

The sense of polarisation is indicated by the colour of the spectrum, since colour is the only extra dimension available in an intensity-modulated frequency-time representation. The recording is made on a 16 mm colour reversal film. If the emission is highly elliptical, the amplitudes of the RH and LH components are almost equal, and the resultant colour is yellow. For highly circular emissions in the RH or LH sense, the spectrum appears green or red, respectively. Slight variations in colour can be detected faster and with greater confidence than small density differences in two parallel black-and-white records.

The observations were carried out from August 1 to November 17, 1974, for 2 h each night at Kiiminki, 30 km east of Oulu. Other spectrographs of the sweep-frequency type were operated simultaneously. Only the multichannel observations are described here. Some interference was caused by residual Faraday fringes. Because of the relatively low altitude of Jupiter (15–18°), the effect of ground reflection could not be avoided. Thus the Faraday rotation modulated the axial ratio of the polarisation ellipse and the relative amplitudes of the circular components.

Sample records reproduced in black-and-white are shown in Fig. 1. Prominent features in the spectra are the radiation areas (envelopes). The modulation lanes traverse them diagonally in a continuous manner. According to the classification given previously², *a* and *b* represent bursts of types L2 and L4, respectively. In the same notation L1 indicates a burst with no detectable lane structure, and L3 a burst in which the positive and negative lanes are superposed.

The durations of the envelopes are related to the elongation of the planet; towards opposition the durations tend to increase². Accordingly, the duration of envelopes in Fig. 1*b* are longer than those in Fig. 1*c*. 'Fast-drift lanes' are observed occasionally, with frequency-time slopes an order of magnitude greater than those of the modulation lanes (Fig. 1*d*). Similar events have been recorded by Ellis^{5,13}.

An occurrence histogram of bursts of types L1 to L4 is presented in Fig. 2. This is constructed in the same way as that for the 1966–69 observations³. Again, type L4 bursts are associated chiefly with regions A and C, and type L2 bursts with region B. But the number of L1 bursts relative to bursts of other types is greater than before, the number of L2 and L3 bursts is less, and bursts of type L3 occur chiefly at region A rather than at region B. The region designation is that of Carr, *et al.*¹⁴.

The result of a careful search for polarisation diversity effects indicates that diversity does not occur as a rule in the spectra of modulation lanes. Most lanes are simple variations in intensity of emission, and thus are similar in appearance in both circular modes. The polarisation is elliptical in the RH sense and the amplitude of the RH component is the larger of the two.

There are a few cases in which diversity does appear. The sense of polarisation is then LH, and may be recognised easily from the colour. Polarisation diversity seems to occur in areas where the amplitude pattern of the spectrum is more complex than just a simple set of lanes. In two such spectra obtained it seems that the LH component may belong to one of the two sets of lanes of the L3 burst. Not all of the L3 bursts, however, display polarisation diversity.

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Wear of synthetic diamond when grinding ferrous metals

It is well established that diamond is an inefficient abrasive for grinding ferrous materials, especially the low carbon variety. The widely held view is that as ordinary carbon has a strong affinity for iron, forming iron carbide (Fe₃C), this should also be the case with diamond (cubic carbon) when grinding steel. The high rate at which diamond grinding wheels wear down when grinding low carbon steel is said to be a consequence of this action. We suggest here another mechanism, evidence of which is provided by scanning electron micrographic (SEM) data.

To simulate grinding and at the same time eliminate the influence of the bonding material, cutting tests have been performed using a single diamond particle mounted on the periphery of a 20 cm (8 inch) diameter aluminium disk. A groove was cut in a soft, low carbon, steel plate (AISI 1018 steel) by moving the flat plate into the rotating diamond particle at a speed *v*. This method of simulating the action of an abrasive particle in the surface of a grinding wheel is termed overcut fly milling^{1,2}. The machine settings—table speed, *v*: 30 cms⁻¹ (12 inches min⁻¹); downfeed (depth of groove), *d*: 25 μm (0.001 inch); and disk speed, *V*: 20.3 ms⁻¹ (4,000 feet min⁻¹)—were chosen to give a chip size comparable to that used in fine surface grinding using a diamond grinding wheel (resulting in an undeformed chip thickness of a few microns). A well developed, single crystal, synthetic (cubo-octahedral) diamond was chosen for study. This type of diamond was

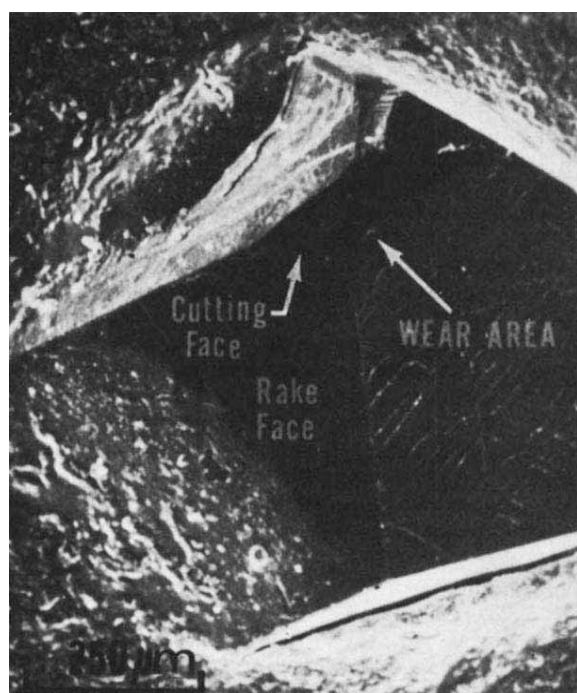


Fig. 1 Micrograph of the wear area of a diamond grit.

selected in preference to a polycrystalline specimen because it is considerably stronger and less inclined to chip by intercrystalline fracture. This combination of tool and method precluded diamond wear by chipping.

Several thousand cuts were made on the work material with each diamond particle tested. After a test, the worn diamond surface was coated with a thin layer of gold-palladium (to overcome the charging problems otherwise experienced in the scanning electron microscope) and the wear areas were examined.

Figure 1 is a micrograph of the worn area of a diamond grit examined in the secondary electron mode. Figure 2 is a micrograph of the worn area obtained by tilting the sample favourably and examining it in the back scatter mode. The worn area is not plane but has a concave shape in a direction transverse to the direction of cut. Figure 3 shows the groove-like markings evident in Fig. 2 at higher magnification. These markings result from a transverse saw tooth pattern (Fig. 4). No wear was evident on the rake face of the diamond across which the chip flows under high pressure.

All of the results described here are consistent with a mechanism of wear in which diamond is converted to graphite before its removal from the worn surface.

Diamond at room temperature is in a metastable state and will transform to graphite in the proper circumstances. An increase of temperature without a suitable increase in pressure will carry the diamond further from equilibrium and the presence of a properly directed shear stress will promote further the transformation to graphite. The presence of a material that rapidly removes graphite from the surface of the diamond should promote this transformation even more. Hot, low carbon steel is such a material. This latter situation does not arise when grinding aluminium or copper alloys with diamond and, consequently, the rate of wear is negligible in those circumstances.

All of these conditions (high temperature, relatively low pressure, high shear stress and hot iron) are present on the worn area on the clearance face of a diamond abrasive particle that is cutting soft, low carbon steel.

The fact that diamond (unlike graphite) is relatively insoluble even in molten iron (a key point in the synthesis of diamond⁸)

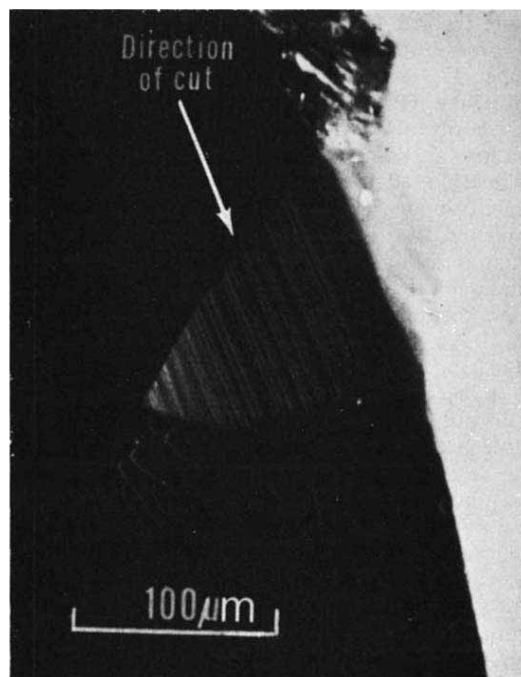
rules out the possibility that diamond reacts directly with iron.

Once diamond graphitises, however, the graphite so formed can rapidly diffuse into iron because of the intimate contact under pressure, the high affinity of graphite for iron and the high interfacial temperatures. Also, the fact that diamond is worn more rapidly by a soft, low carbon steel than it is by a strong, highly alloyed steel, tends to rule out oxidation as the major cause of diamond wear since the alloy steel would give rise to a higher grinding temperature.

The grooves evident in Figs. 2 and 3 could not have been produced by direct abrasive action of any constituent in the soft steel being ground. Instead, those grooves must result from a preferential transformation on 110 planes, as shown in Fig. 4. Prins *et al.*⁴ have reported similar markings on diamonds used to grind hardened tool steel (AISI M42) but were unable to explain their presence. Experiments conducted by Seal⁵ has shown that the 001 faces of diamond were the most resistant to high temperature thermal graphitisation whereas the 110 planes were the least resistant. Bovenkerk *et al.*⁶ have reported that diamonds grow with cubic habit when the conditions of temperature and pressure are almost minimal for a particular catalytic-solvent material, but that they grow with octahedral habit when the temperature and pressure are very much higher. As Seal⁵ has pointed out, the directions of fastest graphitisation are the same as those of the fastest growth, and the directions of slowest graphitisation coincide with those of slowest growth in the lower pressure part of the region of diamond synthesis. These striking similarities suggest strongly that the catalytic-solvent effect of iron, and the concomitant conditions of high temperature and lower contact pressures (relative to the stable region of the diamond) when grinding low carbon steel with diamond, play a very significant role by causing rapid, preferential graphitisation of diamond, leading to poor grinding performance.

The sharp lines evident in Figs. 2 and 3 on the worn areas are approximately parallel but do change direction and intersect occasionally. These lines are thought to be the ends of what Tolansky⁷ refers to as slip planes (found in man made diamonds) which are akin to the foliation markings in natural stone that has been subjected to plastic flow under enormous pressure. It is of interest to note that the spacing of these slip

Fig. 2 Micrograph of the same wear area obtained by tilting the sample and examining in the back-scatter mode.



⁶ Bovenkerk, H. P., Bundy, F. P., Hall, H. T., Strong, H. M., and Wentorf, R. H., *Nature*, **184**, 1094 (1959).
⁷ Tolansky, S., *Proc. R. Soc.*, **A263**, 31 (1961).

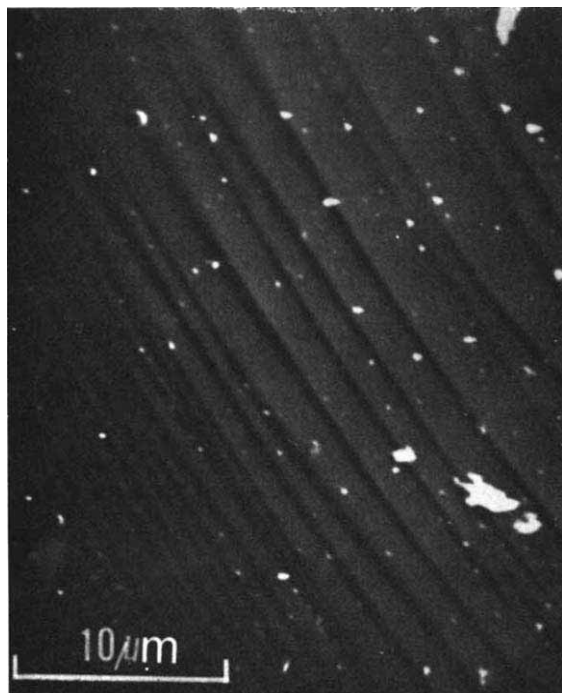


Fig. 3 Groove-like markings in the wear area of diamond grit.

planes is from 1 to 3 μm in the samples of diamond used in this investigation.

The concave shape of the general wear surface is also consistent with the graphitisation theory of wear presented here. The temperature in the central region of the cut will be higher than at the edges and this should give rise to a higher rate of graphitisation in the former region.

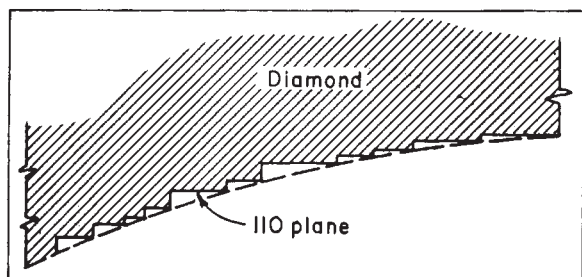


Fig. 4 Graphical representation of saw-tooth markings shown in Fig. 3.

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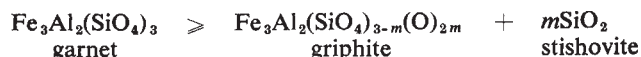
Chemistry and structure of high pressure phase of garnets rich in almandite

I HAVE previously reported^{1,2} high pressure phase transformations for garnets of the almandite-pyrope ($\text{Fe}_3\text{Al}_2\text{Si}_3\text{O}_{12}$ – $\text{Mg}_3\text{Al}_2\text{Si}_3\text{O}_{12}$). Here, the chemical formula and the crystal structure for the high pressure phase of almandite-rich garnets quenched from a loading pressure of approximately 120×10^5 kPa and temperatures of about 1,400–1,800 °C are given.

Details of the samples and experimental procedures have been described elsewhere^{3,4}. Fine polycrystalline powder samples of synthetic almandite and natural almandite-rich garnets were compressed in a diamond anvil press fitted with a lever and spring assembly and were irradiated by a continuous wave YAG laser. The loading pressures of the sample at the central portion of the anvil were estimated from the length of the spring which was calibrated according to the room temperature NaCl pressure scale. The real pressure to which the sample was subjected is probably $\sim 40 \times 10^5$ kPa greater than the loading because of a transient increase of pressure during heating. The temperatures of the samples could be estimated using an optical pyrometer but the uncertainties would be rather large.

The quenched samples have been X rayed with $\text{CoK}\alpha$ radiation in a modified 57.3-mm Debye–Scherrer camera. The resultant diffraction data have been listed in Tables 1 and 2 for the synthetic almandite–garnet and the natural almandite-rich garnet. With the exception of the reflection at $d = 2.95$ Å, nearly 40 observed d -spacings for the high pressure product from synthetic almandite can be indexed on the basis of a structure isotypic with the hydrogarnet, griphite^{5,6}. Many reflections such as 2.866, 2.559, 2.444 Å . . . , which seemed initially to be those of the starting material, almandite, can equally well be derived from the new high pressure phase. This is evident from comparison of the observed d -spacings with those calculated for almandite and for the griphite isotype. The calculated lattice parameter for the high pressure phase with the griphite structure is $a_0 = 11.470 \pm 0.025$ Å. The space group may be $\text{P2}_1/\text{a}\bar{3}$ (ref. 6). The reflection of 2.95 Å may correspond to the most intense reflection (110) of stishovite. Thus, almandite seems to disproportionate into a mixture of stishovite plus a phase with the griphite structure at approximately 120×10^5 kPa loading pressure and 1,400–1,800 °C. With the exception of a very weak unidentified line, 2.071 Å, the high pressure phases for the natural almandite-rich garnet (Table 2) are almost identical to those of the synthetic almandite with the lattice parameter for the griphite structure $a_0 = 11.465 \pm 0.030$ Å.

Griphite is a hydrophosphate garnetoid. Its chemical formula represents a complex example of a general form of $\text{X}_3\text{Y}_2(\text{Z}\text{O}_4)_{3-m}(\text{OH})_{4m}$ with $m = 0.5$ (ref. 5). Because no water was involved in this study of phase transformations of garnet², one would expect the general formula for hydrogarnets to be modified to suit the possible chemical formula of the high pressure phase of almandites. Removing water completely, $2m(\text{H}_2\text{O})$, from the general form of hydrogarnet yields another general formula of $\text{X}_3\text{Y}_2(\text{Z}\text{O}_4)_{3-m}(\text{O})_{2m}$. If the high pressure phase with griphite structure has this formula, then the high pressure disproportionation of almandite can be represented as



Stishovite is indicated by the diffraction patterns (Tables 1 and 2), confirming the proposed reaction. The precise value for

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¹ Brecker, J. N., Komanduri, R., and Shaw, M. C., *Ann. C.I.R.P.*, **22/2**, 219 (1973).

² Komanduri, R., and Shaw, M. C., *Nature*, **248**, 582 (1974).

³ Hall, H. T., US Patent No. 2947608, US Patents Office, Washington, DC, (1960).

⁴ Prins, J. F., Harding, D. R., and James, G. F., *Diamond Research 1970*, 11 (Industrial Diamond Information Bureau, London, 1970).

⁵ Seal, M., *Phys. Status. solidi*, **3**, 658 (1963).

Table 1 Room temperature and 1 bar pressure X-ray diffraction data of synthetic almandite quenched from a loading pressure of approximately 120×10^5 kPa and 1,400–1,800 °C (CoK α).

I/I_{100}^*	Observed $d(\text{\AA})$	Almandite $d(\text{\AA})^\dagger$	hkl	Griphite $d(\text{\AA})^\ddagger$	hkl	$d(\text{\AA})$	Stishovite§ hkl	I/I_{100}
20	4.67			4.68	211			
5	3.47			3.46	311			
5	3.33			3.31	222			
15	3.07			3.07	321			
15	2.95					2.95	110	100
80	2.866	2.883	400	2.868	400			
?	2.78			2.78	410,322			
100	2.559	2.579	420	2.565	420			
40	2.444	2.459	332	2.445	332			
50	2.341	2.354	422	2.341	422			
40	2.247	2.262	510	2.249	510,431	2.24	101	30
40	2.210			2.207	511,333			
50	2.090	2.105	521	2.094	521	2.09	200	1
25	2.020	2.039	440	2.028	440			
—	—					1.98	111	40
5	1.936			1.939	531			
15	1.915			1.912	600,442			
50	1.860	1.871	611	1.861	611,532	1.87	210	20
5	1.815			1.814	620			
?	1.72			1.729	622			
40	1.656	1.665	444	1.656	444			
10	1.622			1.622	710,543			
70	1.590	1.599	640	1.591	640			
15	1.561			1.561	721,633			
80	1.533	1.541	642	1.533	642	1.530	211	70
—	—					1.476	220	30
30	1.435	1.442	800	1.434	800			
10	1.417			1.423	810			
5	1.396			1.412	811			
				1.401	733			
15	1.361			1.391	820			
				1.371	653			
				1.352	822,660			
—	—					1.333	002	10
30	1.281	1.289	840	1.282	840	1.322	310	5
40	1.253	1.258	842	1.252	842			
—	—					1.235	301	25
25	1.223	1.229	664	1.223	664			
5	1.212			1.216	922			
15	1.159	1.165	941	1.209	930	1.215	112	10
5	1.126	1.131	862	1.159	941	1.159	320	10
30	1.0678	1.0707	864	1.125	862			
				1.0696	953			
30	1.0500	1.0527	10,4,2	1.0650	864			
20	1.0173	1.0193	880	1.0471	10,4,2			
—	—			1.0138	880			
10	0.9608	0.9610	12,0,0	0.9625	965	0.9900	222	5
20	0.9480	0.9479	12,2,0	0.9493	12,1,1			
				0.9460	11,5,1			
30	0.9356	0.9354	12,2,2	0.9365	11,5,2			

*Estimated visually. The question mark denotes extremely weak lines.

 † Observed in a standard Debye-Scherrer film with the exception of the last three reflections. d -spacings were calculated on the basis of $a_0 = 11.532$ Å reported by Takahashi and Liu³. ‡ Calculated from $a_0 = 11.470$ Å (see text). § From Chao *et al.*⁷.

m is not known at present, but the maximum value can be calculated since

$$V^0_{\text{almandite}} \geq V^0_{\text{griphite}} + m V^0_{\text{stishovite}}$$

$$(115.45 \text{ cm}^3 \text{ mol}^{-1}) \quad (113.60 \text{ cm}^3 \text{ mol}^{-1}) \quad (14.03 \text{ cm}^3 \text{ mol}^{-1})$$

where V^0 is the standard molar volume. So $m \leq 0.13$. The observed relative intensities for griphite and stishovite in Tables 1 and 2 support this estimate. If $m = 0.10$, the volume change associated with the disproportionation of almandite is -0.4% .

The quantity of stishovite in the products is expected to be very small, the evidence of its presence is rather weak since

only one reflection line has been found to correspond to the most intense line of stishovite. An alternative explanation would be that this weak line (2.95 Å) may be derived from one of the intermediate phases between the griphite and the perovskite structures^{1,2}. The observed griphite form may, therefore, be a stoichiometric $X_3Y_2(ZO_4)_3$ compound with the associated volume change for the polymorphic phase transition being -1.6% .

The high pressure phase of griphite structure found in this study is probably formed within a pressure range of $100\text{--}150 \times 10^5$ kPa. This pressure can be created naturally by meteorite impact, which seems to be the origin of mineral stishovite. Rocks containing a high proportion of almandite, such as mica and other schists, that have been subjected to

Table 2 As Table 1, for natural almandite*

I/I_{100}	Observed d (Å)	Natural almandite d (Å)	hkl	Griphite d (Å)	hkl	d (Å)	Stishovite hkl	I/I_{100}
15	4.70			4.68	211			
15	3.45			3.46	311			
10	3.23			3.31	222			
				3.18	320			
10	3.06			3.06	321			
15	2.96					2.95	110	100
80	2.864	2.881	400	2.866	400			
5	2.80			2.78	410,322			
100	2.564	2.577	420	2.564	420			
40	2.445	2.457	332	2.444	332			
50	2.342	2.352	422	2.340	422			
45	2.247	2.260	510	2.248	510,431	2.24	101	30
40	2.210			2.206	511,333			
45	2.095	2.104	521	2.093	521	2.09	200	1
5	2.071†							
20	2.030	2.037	440	2.027	440			
?	1.97			1.966	530	1.98	111	40
50	1.861	1.869	611	1.860	611,532	1.87	210	20
15	1.814			1.813	620			
15	1.732			1.748	533			
				1.728	622			
40	1.657	1.663	444	1.655	444			
5	1.621			1.621	710,543			
70	1.592	1.599	640	1.590	640			
10	1.564			1.560	721,633			
80	1.534	1.540	642	1.532	642	1.530	211	70
—	—					1.476	220	30
30	1.436	1.440	800	1.433	800			
10	1.416			1.422	810			
				1.411	811			
5	1.395			1.401	733			
				1.390	820			
15	1.368			1.370	653			
—	—					1.333	002	10
25	1.283	1.288	840	1.282	840	1.322	310	5
30	1.253	1.257	842	1.251	842			
—	—					1.235	301	25
20	1.224			1.222	664			
10	1.210			1.209	930	1.215	112	10
15	1.160	1.164	941	1.158	941	1.159	320	10
10	1.125	1.130	862	1.124	862			
40	1.0676	1.0699	864	1.0691	953			
				1.0645	864			
30	1.0500	1.0519	10,4,2	1.0554	10,3,3			
				1.0466	10,4,2			
30	1.0172	1.0185	880	1.0214	10,5,1			
				1.0134	880			
—	—					0.9900	222	5
10	0.9596	0.9603	12,0,0	0.9621	965			
				0.9554	12,0,0			
15	0.9476	0.9472	12,2,0	0.9489	12,1,1			
				0.9456	11,5,1			
20	0.9347	0.9346	12,2,2	0.9361	11,5,2			

*See footnotes of Table 1 with the exception that the d -spacings for natural almandite were calculated from $a_0 = 11.523$ Å reported by Takahashi and Liu³ and those for griphite structure from $a_0 = 11.465$ Å.

†Unidentified line.

impact may yield minor quantities of the new griphite phase.

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Benthic nutrient regeneration and its coupling to primary productivity in coastal waters

SHALLOW, near-shore ocean waters support high primary production because of the availability of inorganic nutrients. The availability is usually attributed to the proximity of freshwater runoff or to coastal upwelling and deep water advection^{1,2}.

The effects of nutrient regeneration from the ocean bottom have not, however, been estimated. We report here the first successful attempts to measure directly the nutrient flux from

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² Liu, L., *Earth planet. Sci. Lett.*, (in the press).

³ Takahashi, T., and Liu, L., *J. geophys. Res.*, **75**, 5757–5766 (1970).

⁴ Liu, L., *Earth planet. Sci. Lett.*, **24**, 357–362 (1975).

⁵ McConnell, D., *Am. Mineral.*, **27**, 452–461 (1942).

⁶ Peacor, D. R., and Simmons, Wm. B., Jr., *Am. Mineral.*, **57**, 269–272 (1972).

⁷ Chao, E. C. T., Fahy, J. J., Littler, J., and Milton, D. J., *J. geophys. Res.*, **67**, 419–421 (1962).

nearshore sediments. We contend that the continental shelf itself is a tightly coupled component of most near-shore ecosystems and that the function of the shallow sea floor in nutrient regeneration has been underestimated in previous investigations.

Nitrogen is considered to be the element limiting photosynthesis in coastal waters³. Although it has been presumed that the bottom is an important site of nitrogen remineralisation⁴, attempts to estimate benthic regeneration have been confined to lakes^{5,6}, indirect inferences from concentrations^{7,8} of pore water, or laboratory incubations which indicate that the bottom is only a minor source of nutrients⁹ or even a nitrogen sink in anoxic areas¹⁰.

It is necessary to know the rate at which ammonia and nitrate diffuse into the water column from the sediment to determine the degree of coupling between the bottom and primary production in the water. An indirect method which we have used is the assumption that the breakdown of organic matter in sediment is proportional to the amount of oxygen required by the sediment^{11,12} although that probably, to some degree, underestimates total catabolism¹³. For each millilitre of oxygen consumed, we assumed that 0.412 mg organic carbon was oxidised to CO₂. The organic carbon-nitrogen ratio in sediments is about 10:1, or greater, and therefore we assumed that 0.041 mg organic nitrogen would be remineralised to ammonia for each millilitre of O₂ consumed. Numerous measurements of the amount of oxygen required by the sediment have been made and it is, therefore, relatively easy to

estimate the degree of ammonia production of the bottom in many regions. The oxygen demand of the sediment in the sublittoral zone ranges from about 1 to over 100 ml m⁻² h⁻¹ (ref. 12) and, therefore, 0.041–4.1 mg NH₄ m⁻² h⁻¹ (2.0–292 µg-atom NH₄) should be produced by excretion.

We presumed that if the predicted regeneration occurred it would diffuse out of the bottom. We therefore carried out *in situ* experiments to test this. An opaque, 7.5-l bell jar, with a stirring motor and an O₂ electrode¹⁴, was used to incubate a 730 cm² area of the bottom for various lengths of time. (The stirrer circulates the water at a velocity somewhat less than natural tidal currents—up to 15 cm s⁻¹—and it maintains exchange over the electrode.) Periodically, we withdrew water samples from the jar and measured the concentrations of ammonia, nitrate, nitrite and phosphate accumulating within the enclosure¹⁵.

The amount of ammonia evolved was quite large (Table 1). Both the oxygen demand of the sediment and ammonia production were highly dependent on temperature. The November experiment came closest to a yearly average temperature and to what we would expect is an average oxygen demand (22.08 ml m⁻² h⁻¹), corresponding to a production of 0.91 mg N (64.6 µg-atom m⁻² h⁻¹), which was close to that actually measured (69 µg-atom m⁻² h⁻¹) evolving into the bell jar. Most of the nitrogen at warmer temperatures was in the form of ammonia. At lower temperatures, some had been oxidised to nitrite and nitrate, suggesting there may have been loss of nitrogen from the system by denitrification to N₂ (ref. 10). On average, 81% of the nitrogen predicted to return to the water did so. Possible sources of error were nutrient uptake by plants in the bell jar, lower-than-normal diffusion out of the mud, or ammonia binding by cation exchange to clay particles¹⁶.

Because nutrients from freshwater could not account for the high near-shore productivity, Riley¹ proposed a model which moved deep nutrients onshore by horizontal advection and tidal mixing. This mechanism is not needed to account for the productivity gradient if benthic feedback (recycling) is included in the system. Bottom respiration on the continental shelf should be near 10–20 ml m⁻² h⁻¹ (refs 11, 12, 14 and 17). This means that 4.12 mg organic carbon and 0.412 mg organic nitrogen (29.4 µg-atom N) would be oxidised to CO₂ or deaminated to NH₄⁺, respectively. If about 80% of the NH₄⁺ were released from the sediment, the average feedback for the continental shelf would be about 23.5 µg-atom N m⁻² h⁻¹.

We cannot yet confirm this by *in situ* measurements on offshore, continental shelf sediments, though there is strong evidence that our indirect estimates are close to reality (ref. 3, and N. Corwin, unpublished). Ammonia concentrations at continental shelf locations in late summer 1969, south of Long Island (Fig. 1) increased by about 2.5 µg-atom l⁻¹ in the bottom 10–15 m of the water column. This gradient was probably maintained by photosynthesis below the thermocline and by vertical eddy diffusion. The flux along the gradient can be represented by a finite difference equation:

flux of ammonia = K (change in concentration/change in depth)

where K is a 'mixing constant' with values ranging from <1 to >100 (cm² s⁻¹)¹⁸. Over a typical sand bottom (Stations 1502, 1503, 1510, Fig. 2) the average near-bottom gradient was about 0.2 µg-atom NH₄⁺ l⁻¹ m⁻¹. Using 2 cm² s⁻¹ for K (see ref. 18) we estimate that 144 µg-atom NH₄⁺ m⁻² h⁻¹ evolved from the sediment in order to maintain the gradient. The profiles from stations at the New York sewage sludge dump (1504, Fig. 1) and the Hudson Shelf trough had higher bottom gradients. Oxygen demand there at a temperature of 13 °C was 43 ml O₂ m⁻² h⁻¹ (ref. 17), equivalent to a regeneration of 126 µg-atom N m⁻² h⁻¹. The low ammonia concentrations off the continental shelf (Station 1512, Fig. 2) are evidence that the high concentrations of ammonia on the bottom of the shelf did not arise from advection from deep offshore water. Because an intense thermocline existed when these samples were taken,

Fig. 1 Concentrations of ammonia in the water column at selected locations on the continental shelf off New York, September, 1970 (ref. 3 and N. Corwin, unpublished). Stations 1504 and 1505 are the New York City sewage sludge dump site and the Army Corps of Engineers dredge spoils dump site, respectively. The terminal datum point on each profile is from the bottle, several metres above the bottom.

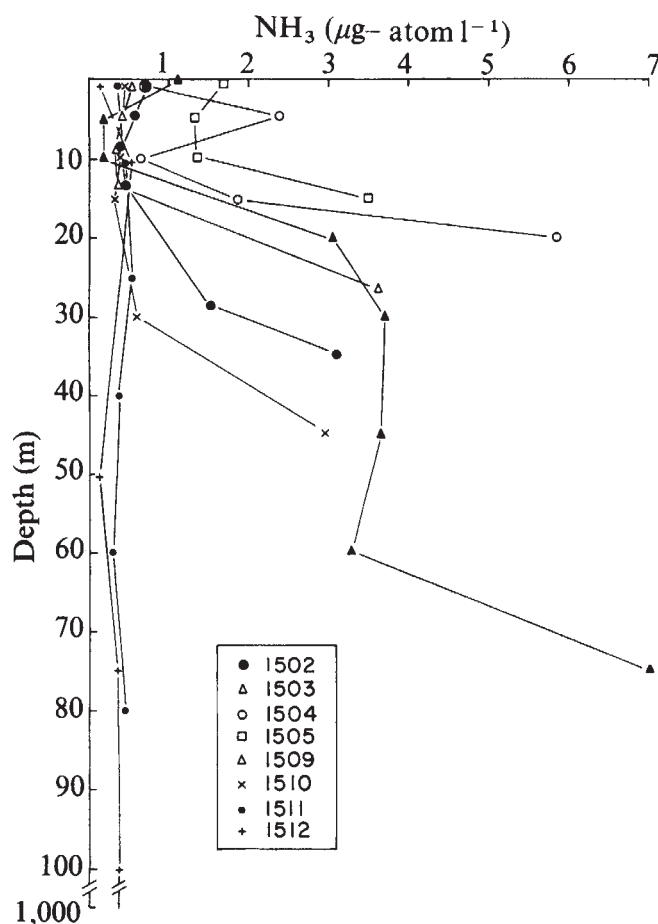


Table 1 Sediment-water nutrient flux

Location and depth	Date	Nutrient flux ($\mu\text{g-atom m}^{-2} \text{ h}^{-1}$)					Bottom O_2 demand ($\text{ml m}^{-2} \text{ h}^{-1}$)	Predicted N production	Observed N as % of predicted N	Mean O_2 Concentration (ml l^{-1})	Duration (h)	T ($^{\circ}\text{C}$)
		NH_4^+	NO_2^{2-}	NO_3^{3-}	ΣN	PO_4						
Buzzards Bay (17 m)	November 1973	68.8	0.05	0.15	69.00		22.08	64.9	106	4.8	2.15	7.7
Buzzards Bay (17 m)	January 1974	2.56	4.28	4.79	11.63	4.28	10.90	32.1	36	7.8	6.00	1.5
Buzzards Bay (17 m)	February 1974	18.70	1.19	1.19	21.08	5.17	10.35	30.4	69	8.3	2.6	1.6
Buzzards Bay (17 m)	June 1974	124.0	-1.96	2.93	124.97	-14.87	41.89	123.3	101	4.7	5.25	16.0
Eel Pond (2 m)	July 1973	7.87	0.44	1.75	10.06	6.6	14.96	44.0	23	6.2	4.5	20
		117.99	0.94	-1.50	117.43	21.57	51.59	151.8	77	4.7	10.5	20
		127.91	0.46	0.0	128.37	19.46	26.88	79.1	162	2.7	13.0	20
Eel Pond Average		84.59			85.29		31.14	91.6	93			

the ammonia could not have come from Hudson River effluent, because that water would have remained mostly near the surface. The high ammonia content must therefore have come from regeneration on or near the bottom.

We can assess the significance of this recycling in two ways. The area of the New York Bight (about $1.17 \times 10^{10} \text{ m}^2$) would recycle $16.8 \times 10^{11} \mu\text{g-atom NH}_4^+ \text{ h}^{-1}$, if our estimated flux of $144 \mu\text{g-atom NH}_4^+ \text{ m}^{-2} \text{ h}^{-1}$ is correct. On the other hand, the Hudson River and Raritan estuary contribute $8.6 \times 10^{10} \mu\text{g-atom h}^{-1}$, if one assumes that the net contribution of freshwater, averaged over one year, is $2.4 \times 10^9 \text{ l hr}^{-1}$ (ref. 19), and that the average ammonia concentration for the river water is $35.97 \mu\text{g-atom NH}_4^+ \text{ l}^{-1}$ (N. Corwin, unpublished). Another important comparison concerns the nitrogen requirements for photosynthesis. The average primary production rate for Stations 1502, 1503, 1509, 1510 and 1511 (Fig. 2) was $0.150 \text{ g C m}^{-2} \text{ d}^{-1}$ (N. Corwin, unpublished), and if the nitrogen used was about 15% of this, the nitrogen requirement for photosynthesis was

$67 \mu\text{g-atom m}^{-2} \text{ h}^{-1}$. Based on our estimate ($144 \mu\text{g-atom m}^{-2} \text{ h}^{-1}$), bottom regeneration at the time the data were taken could be supplying more than the total required nitrogen. If there were no contribution from the bottom sediments the system would lose this feedback and the production of plant biomass, dependent only on water column regeneration, would be reduced to rates similar to those found beyond the continental shelf.

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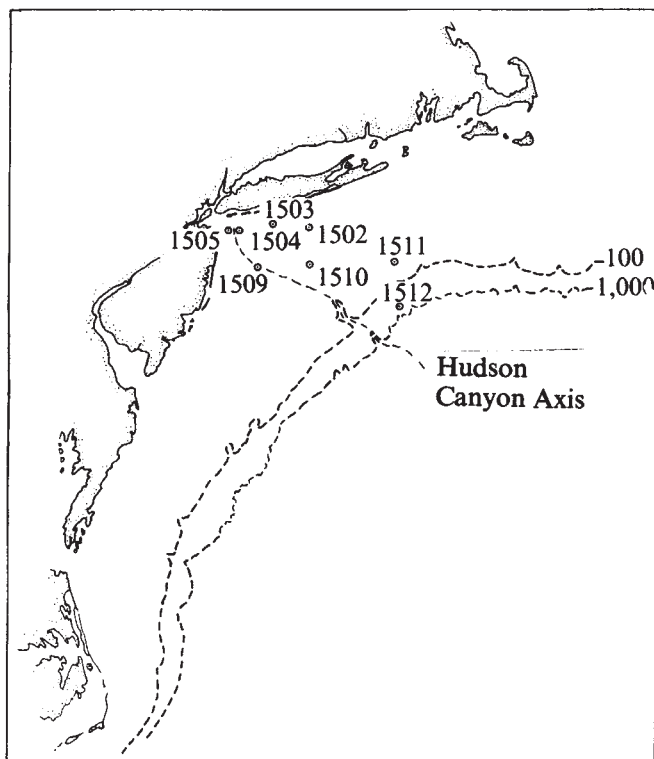
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Fig. 2 Locations of hydrographic stations shown on Fig. 1. Dashed lines parallel to shore are 100 and 1,000 fathom isobaths.



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Chemical alkylation of lead

FOLLOWING the reported methylation of lead by anaerobic microorganisms¹ we have investigated organolead compounds in aqueous systems. Our results indicate the possible existence of chemical mechanism for the conversion of trimethyl lead acetate, Me_3PbOAc , to tetramethyl lead (TML) in active anaerobic sediments.

Two basic systems were studied. A 'sulphide' system comprising sodium sulphide (hydrated, 15 mg) in distilled

water (200 ml) and a 'sediment' system comprising an anaerobic lake sediment (50 ml), a nutrient broth (CM1), (1 g) and glucose (0.2 g) made up to 200 ml with distilled water. All of the systems were sealed by Subaseals in 250 ml volumetric flasks and stored in the dark. The atmosphere above the solutions was sampled by a syringe and tested for tetraalkyl leads by direct injection into a gas liquid chromatography column coupled to an electron capture detector. The identities of the tetraalkyl leads produced were ascertained by comparison of their retention times with those of authentic samples.

The addition of Me_3PbOAc (15 mg) to a sediment system produced detectable amounts of TML in the atmosphere above the solution after 1 d and the concentration built up gradually to $6\text{ }\mu\text{g ml}^{-1}$ over two weeks. This gives an approximate figure of $300\text{ }\mu\text{g}$ for the total TML in the atmosphere, which compares favourably with the previously reported figures¹ ($125\text{ }\mu\text{g}$ after one week and $642\text{ }\mu\text{g}$ after two weeks), considering the unknown amount of TML absorbed in the solution and on to the walls of the vessel.

The addition of Me_3PbOAc (15 mg) to a sulphide system produced TML within 30 min and the concentration built up to $0.5\text{ }\mu\text{g ml}^{-1}$ in 7 h and $6\text{ }\mu\text{g ml}^{-1}$ in 2 d. The addition of Me_3PbOAc to distilled water with no added sulphide gave a constant background level of TML of $0.01\text{ }\mu\text{g ml}^{-1}$. Substitution of trimethyl lead chloride (Me_3PbCl) for Me_3PbOAc gave identical results.

The addition of 15 mg of triethyl lead chloride (Et_3PbCl) to a sediment system produced tetraethyl lead (TEL) in the atmosphere above the solution. This was detectable after 4 d at a concentration of $0.003\text{ }\mu\text{g ml}^{-1}$. In this time no methyltriethyl lead (MeEt_3Pb) was detected. The addition of Et_3PbCl to a sulphide system produced TEL to a concentration of $0.016\text{ }\mu\text{g ml}^{-1}$ in 4 d. A solution of Et_3PbCl (15 mg) in distilled water (200 ml) produced no detectable TEL in the atmosphere above it.

A sediment system to which no lead had been added produced no detectable tetraalkyl leads. We were also unable to detect any TML from the addition of lead nitrate ($\text{Pb}(\text{NO}_3)_2$) to sediment systems.

These results suggest that the main reaction producing tetraalkyl lead from trialkyl lead salts in anaerobic systems is the conversion of the trialkyl lead salt to the sulphide, and the subsequent decomposition of the trialkyl lead sulphide ($\text{R}_3\text{Pb})_2\text{S}$ to form the tetraalkyl lead. The absence of Et_3MePb when Et_3PbCl is added to sediment shows that no direct methylation is occurring in this case.

Sulphide, in the form of hydrogen sulphide is liberated by many organisms under anaerobic conditions. This would normally react with any metallic ions present to form insoluble sulphides (such as, ferrous sulphide) but, presumably, the trialkyl lead salts compete with these inorganic ions for the sulphide. An earlier report¹ stated that no TML was produced after autoclaving of the sediment; in such a system, the amount of free sulphide available will be low because of the reaction of any H_2S present to form insoluble sulphides, and further H_2S will not be made available for the trialkyl lead salt because of lack of biological activity. Thus, if the mechanism proposed here is correct, production of TML from Me_3PbOAc would not be expected to proceed after autoclaving of the sediment.

Furthermore, we have shown that one of the major routes in the methylation of mercury in biological systems is not available to these lead compounds. Following the method of Bertilsson and Neujahr² for the methylation of inorganic mercury by methylcobalamin, we substituted equimolar quantities of Me_3PbOAc , Me_3PbCl , Me_3PbCl_2 and $\text{Pb}(\text{NO}_3)_2$ for the inorganic mercury. In none of these cases did methylation occur.

We propose, therefore, that a possible mechanism for the production of TML from Me_3PbOAc in anaerobic systems is the initial formation of $(\text{Me}_3\text{Pb})_2\text{S}$ followed by decomposition to give TML as one product. This mechanism seems to be

dominant in the reaction of Et_3PbCl in anaerobic conditions to form TEL.

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Accuracy of dates beyond the ^{14}C dating limit using the aspartic acid racemisation reaction

RECENT evidence suggests that the racemisation reaction of aspartic acid can be used to date fossil bones^{1–3} (for a general review, see ref. 4). At 20°C , the half life for aspartic acid racemisation is about 15,000 yr. Because the aspartic acid reaction has a longer half life than radiocarbon, it can be used to date bones which are beyond the ^{14}C dating limit (that is, $>40,000$ yr old). Also, only a few grams of bone are required for a DL-aspartic acid determination, so bones which are available in insufficient quantities for radiocarbon dating can be dated from the extent of aspartic acid racemisation.

The technique is best applied by 'calibrating' the rate of racemisation reaction, using the extent of racemisation in a bone which has been dated by radiocarbon^{1–3}. Once this calibration has been carried out for a particular site, then other bones from the site can be dated based on the extent of their racemisation. Ages deduced using this technique have been shown to be in close agreement with radiocarbon ages^{1–3}.

It is assumed when using this calibration procedure that the average temperature experienced by the calibration sample is approximately the same as that experienced by the sample which is being dated; a 1° difference in average temperature causes about a 20% change in the racemisation rate constant. Although this equal temperature assumption is certainly valid when the ages of the calibration and dated samples are nearly the same, there is a question of whether it is true when the sample age is much greater than that of the calibration sample. Previous results have suggested that a bone which has a radiocarbon age of about 18,000–20,000 yr is a suitable calibration sample for dating older bones^{1–3}, particularly those too old for ^{14}C dating. In this paper, we have determined the aspartic acid racemisation ages of several bones which are too old for radiocarbon dating. The aspartic acid ages were deduced using a bone about 17,000 yr old as a calibration sample. The ages derived using racemisation are in very close agreement with the ages deduced from other evidence.

The bone samples used in this study came from the Nelson Bay⁵ and Klasies River Mouth (KRM) caves in Southern Cape Province, South Africa (ref. 6 and R. Singer and J. J. Wymer, unpublished). The KRM caves contain a long sequence of Middle Stone Age horizons. Radiocarbon dates^{7,8} for various South African sites, including the KRM caves themselves, indicate that the end of the Middle Stone Age lies near or beyond the dating limit of radiocarbon (that is, greater than 35,000 or 40,000 yr).

The extent of racemisation of aspartic acid in the bones was determined using the procedures described elsewhere¹; in general, about 5–10 g bone was processed. The DL-aspartic acid ratios and their corresponding ages, the stratigraphic

Table 1 Aspartic acid racemisation results for Nelson Bay and KRM caves

Site*	Stratigraphic unit	Depth below deposit surface (m)	DL-aspartic acid	¹⁴ C age (yr)	Aspartic acid age† (yr)
Nelson Bay cave	YSL‡	1.8	0.151	16,700 ± 240 (I-6516)§	$k_{asp} = 4.92 \times 10^{-6} \text{ yr}^{-1}$
	YGL	2.0	0.167	18,100 ± 550 (UW-185)§	
				18,660 ± 110 (GrN-5889)¶	
KRM cave I	13	1.5	0.370	> 30,000 yr	20,000
	16	1.75	0.467	> 30,000 yr	65,000
	18	2.75	0.474	> 30,000 yr	89,000
	19	3.0	0.548	> 30,000 yr	90,000
					110,000

*The caves are located in close proximity and have the same general climate, so the calibration value for Nelson Bay cave should be applicable to the KRM cave as well.

†See ref. 1 for equation used to calculate k_{asp} value and the aspartic acid ages.

‡Sample used for site calibration.

§Determined on associated charcoal.

¶Determined on associated ostrich eggshell.

||The radiocarbon results for these levels are discussed and listed by Klein⁹.

relationship of the samples, and the radiocarbon dates are summarised in Table 1.

The geological evaluation of the KRM cave I profile (K. W. Butzer, personal communication), oxygen isotopic analysis of marine mollusc shells from the relevant KRM levels (N. J. Shackleton, personal communication), and Klein's faunal studies⁸, strongly suggest that the bulk of the KRM cave I Middle Stone Age sequence was deposited during the last interglacial period. The KRM cave I sequence begins on a raised beach which is 6–8 m above the present sea level (ref. 6 and R. Singer and J. J. Wymer, unpublished). The radiometric dating of fossil corals on Oahu, Hawaii⁹, and elsewhere^{10–12} has indicated that the last time the level of the sea was appreciably higher than it is today was 120,000 yr b.p. This age must represent the maximum age for the KRM cave I deposit, and is in close agreement with the age of 110,000 yr deduced from the extent of aspartic acid racemisation in bones from the lowest occupational horizon at KRM cave I (that is, unit 19).

The Middle Stone Age sequence at the KRM caves contains marine food refuse including seals, birds, fish and shellfish⁸. This indicates that in the time interval during which Middle Stone Age people occupied the caves, the sea was never very far away. (At present, the cave is 10–15 m from the sea.) Following the high sea level stand of 120,000 yr ago, there was a fairly long interval (about 40,000–60,000 yr) of oscillating sea level^{12–16}. Periods when the level of the sea was close to but slightly lower than present levels have been dated^{12–15} at about 105,000 and about 85,000 yr b.p.; during the intervening periods sea level was significantly lower than it is today^{15,16}. These latter high sea level periods coincide extremely well with the ages deduced for the middle and lower Middle Stone Age occupational horizons (that is, units 16, 18, and 19) at KRM cave I.

Klein⁸ states that there is a decrease in the relative proportion of seal and marine bird remains in the youngest Middle Stone Age levels of the KRM caves, including unit 13 of KRM cave I. Following this faunal transition period, the cave was abandoned by Middle Stone Age people and remained uninhabited until about 6,000 yr ago. Klein suggests that this occupational gap coincides with the period of essentially permanent depressed sea level during the height of the last glacial age. Sea level curves given by various workers^{13–16} indicate that this period of low sea level began about 60,000–70,000 yr b.p. The aspartic acid racemisation age of 65,000 yr for unit 13 at KRM cave I thus also agrees well with the age range estimated from the faunal and archaeological evidence.

We believe that these results provide convincing evidence that the aspartic acid racemisation can be used to deduce accurate ages of fossil bone which are beyond the dating limit of radiocarbon.

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Male transfer in olive baboons

STUDIES of human populations have shown that inbreeding results in an increase in the number of offspring with greatly reduced viability. It seems likely that there are similar effects in other primates. In *Papio anubis* it seems that this problem is reduced by the transfer of males between groups. In a population studied at Gombe National Park in western Tanzania, inbreeding may be essentially avoided through the transfer of all males out of their natal troop.

Since 1967, 41 individuals are known to have transferred in or out of three study troops at Gombe. Thirty-nine of these were males, the other two were an adult female and her juvenile daughter. Of the 39 transferring males, two were juveniles at the time of transfer, four were old adults (with worn canines) at their first observed transfer, and the remaining 33 were subadult or mature adults. Of the 18 adult males that currently reside in the three study troops, 13 are known to have transferred in from outside that troop; the other five are all old and could have transferred before research at Gombe began.

In two of the troops, demographic data has been maintained constantly since October 1967, in the third since January 1970. This work was begun by T. Ransom and subsequently continued by L. Taylor-Nash, N. Owens, and A. Sindamwo. Of the 20 oldest subadult males that were

members of the study troops when individuals of those troops were first recognised, 15 are known to have transferred, two disappeared, and three died. None have remained in their natal troops. These 15 transfers all left between the ages of approximately 6–8 years; puberty begins at about 5–5½ years and full size is reached at 6½–7½. Although adults which have not yet transferred may attain a high dominance status among mature males, they consistently rank lowest in time spent consorting oestrous females.

Exchange of males at Gombe involves seven different troops. All transfers have been between troops with adjacent ranges. Six males are known to have transferred more than once. Two of these came back to their natal troop, then returned to the transfer troop. The other four are known to have been members of three troops, while one of these is believed to have been a member of four different troops. Three other males are also believed to have transferred more than once, while all the older migrating individuals may have already switched before their initially observed transfer.

Of the males that have reached maturity since 1967, none has remained in his natal troop. Although it is possible for a female to transfer, it is highly unusual. It seems that every male transfers at some time during his life, most likely spending his reproductive life in a troop other than his natal troop. This phenomenon is still being studied in detail.

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Evolutionary trends in the Indian *Iseilema*

Of the most highly evolved grasses, the most useful to man are chiefly confined to the tropical and the warm temperate regions. For example, the Andropogoneae are extensively cultivated for cereals (*Sorghum* spp.), sugar cane (*Saccharum* spp.), essential oils (*Cymbopogon* spp.), and a variety of other purposes. They have attained high adaptive peaks of evolution and species differentiation in the Indo-Malaysian region¹, where there is a high or moderately high rainfall. Three species of the Indian grass *Iseilema* are cultivated for fodder, each one of which is a 'species complex' or a cluster of chromosome races, not randomly united but formed by the genetic system of the taxonomic species. All three complexes show a gradually ascending series of haploid chromosome numbers from 3 to 18 (Table 1), and even the polyploid numbers form an 'aneuploid' series within a complex. Selection, environment, polyploidy and B chromosomes have had varying roles in the evolution of these complexes, and their most notable characteristic is that they form a self-consistent system of evolutionary mechanisms. Here, I demonstrate how *Iseilema* is indicative of the ancient dibasic polyploidy in its tribe, of which it is the highest evolved member, in a habitat where the tribe shows maximum diversity and closest adaptation.

The higher chromosome numbers (Table 1) of *I. laxum* and *I. prostratum* are probably derived from a lower number or numbers by way of remote amphiploidy. For instance, the haploid numbers 14, 14+1B, 15 and 18 of *I. laxum* can all be traced back in a reticulate manner to four secondary basic numbers such as $x_2=6$, 7, 9 and 10 and the latter in turn can be deduced from $n=3$, 4, and 5 of *I. antheophoroides*. The ancient diploid ($n=4$) that is still lingering in the species complex of *I. laxum* lends support to such a view. Hooker³ stated that *I. antheophoroides* is "nearly allied to *I. laxum*".

In spite of many cycles of polyploidy (the highest known

Table 1 The ascending series of haploid chromosome numbers of three Indian species of *Iseilema**

<i>Iseilema antheophoroides</i> Hack.	3	Ramana Rao ⁴
<i>Iseilema antheophoroides</i> Hack.	3	D'Cruz and Birari ⁵
<i>Iseilema antheophoroides</i> Hack.	3+1B	This paper
<i>Iseilema antheophoroides</i> Hack.	4	This paper
<i>Iseilema antheophoroides</i> Hack.	4+1B	This paper
<i>Iseilema antheophoroides</i> Hack.	5	This paper
<i>Iseilema antheophoroides</i> Hack.	5+1B	This paper
<i>Iseilema antheophoroides</i> Hack.	6	This paper
<i>Iseilema antheophoroides</i> Hack.	6+1B	This paper
<i>Iseilema laxum</i> Hack.	4	Celalier and Paliwal ⁶
<i>Iseilema laxum</i> Hack.	14	Joshi, Patil and Manchanda ⁷
<i>Iseilema laxum</i> Hack.	14	Ramana Rao ⁴
<i>Iseilema laxum</i> Hack.	14+1B	This paper
<i>Iseilema laxum</i> Hack.	15	This paper
<i>Iseilema laxum</i> Hack.	18	Ramanathan ⁸
<i>Iseilema prostratum</i> (Linn.) Anderss.	10	This paper
<i>Iseilema prostratum</i> (Linn.) Anderss.	14	Joshi, Patil and Manchanda ⁷

* Pending a careful biosystematic study to validate the taxonomic characters such as sterility and variation in the relative lengths of the spikelets¹⁵, sometimes caused respectively by B chromosomes and polygenes, Hooker's species³ are still maintained.

to date in the genus), some plants of *I. laxum* ($n=14$) have one B chromosome, and seem to have been selected and retained within the complex, whereas others, with $n=15$ and 18, do not. The B chromosome may play a role in restoring vigour, presumably lost as a result of functional diploidy in these lower polyploids; such a source of vigour, as alternative to polyploidy, is no longer required when the basic number increases and hence the higher polyploids tend to eliminate B chromosomes in *I. laxum*.

I. antheophoroides, however, shows an imposing array of as many as eight different chromosome numbers with one B chromosome at each stage, the presence or absence of which does not affect the chiasma frequency at metaphase I in pollen mother cells. Here, selection is progressing not in favour of an increase but towards constancy in the number of B chromosomes, resulting in balanced polymorphism. In this continuous series of chromosome numbers there is at the moment no way of predicting which, if any, is the most primitive. This difficulty is further enhanced by the fact that several basic numbers have been proposed for the Andropogoneae⁹. If statistics alone can provide an unequivocal answer, then $x=5$, the most frequent number, is an important evolutionary radiation in the Andropogoneae and the same set of five chromosomes is also basic for *Iseilema*. At one time, the Andropogoneae were probably in a state of great flux, centred round a basic set of five chromosomes, from which not only several genera like *Iseilema* but even the highest evolved Maydeae seem to have originated.

Themeda, considered to be phylogenetically related to *Iseilema* (Hubbard and Bor, personal communication), is also based on $x=5$ (refs. 10 and 11). *Themeda* and *Iseilema* may have had a common ancestor with five basic chromosomes. Further, the supposed $x=5$ of *Iseilema* need not be derived from $n=10$, encountered in *I. prostratum* and in other Andropogoneae, through such numbers as $x=9$ and $x=7$ also found in *I. laxum* and *I. prostratum*, because $x=9$ and $x=7$ are in themselves amphiploid and can easily be derived from the $n=3$, 4 and 5 of *I. antheophoroides*; similarly, with $n=6$ which co-exists with the $n=3$.

If $x=n=5$ is accepted as a working hypothesis, the haploid number $n=3$ in *I. antheophoroides* can be derived directly or indirectly from $x=5$ by a progressive and step-wise reduction in the basic number (for example, *Airopsis tenella*¹²), also providing desirable centromere economy. Such a chromosome reduction seems to have gone on unobtrusively in such tribes as Aveneae, Stipeae and Agrostaeae

in parallel and independent lines but not to the extreme extent found in the Andropogoneae. Parallel chromosome reduction in these grass tribes may be indicative of their genetical relationship and common inheritance from the ancestral grass stock, which has emerged¹³ from the Liliaceous stock through Juncales by the process of extreme reduction. Thus, instead of being merely a basic number³, $n=3$ of *I. anthephoroides* represents a highly advanced number, perhaps the culmination in the tropical Andropogoneae.

What is remarkable in *I. anthephoroides* is not the stringent centromere economy as a measure of achieving the long range advantage in evolution, but the acquisition of B chromosomes to compensate for a decrease in the number of normal chromosomes. It may well be that a reduction to such a low number as 3, the lowest known to date in grasses, has become an evolutionary possibility resulting from the material properties of B chromosomes, which probably belong to an ancient lineage. In other words, the B chromosomes seem to act as buffers to the haploid chromosomes and provide a mechanism for an elastic adjustment of the increased nucleic acid metabolism, as demanded by the evolutionary changes, both within and outside the species.

It is clear that *I. anthephoroides* has achieved internal stability by an extreme reduction in the haploid number to 3 and plasticity by still retaining excessive variability in the haploid number from 3 to 6, with one B chromosome at each stage. These are two conflicting but delicately balanced evolutionary needs which seem to be advantageous to the species. It is in fact a compromise imposed by selection.

What do we learn when the chromosomal evolution in *Iseilema* is related to its geographic distribution? With all the advantages that a balanced polymorphism can confer on a species population, *I. anthephoroides* is still confined to the southern Deccan Peninsula. This may be caused by the fact that, along with increasing specialisation, the species has become adapted to a particular set of environmental conditions obtained only in that area; or the B chromosomes have adaptive properties of their own restricting the species to its present range of distribution (for example, *Clarkia lingulata*)¹⁴. In contrast, the species complex of *I. laxum* has combined polyploidy and one B chromosome with adaptability, although its area is not commensurate with the high degree of its ploidy, indicating that at least some of its chromosome sets are probably not qualitatively very different from each other. It is distributed over the entire Deccan Peninsula and in Ceylon with a peripheral distribution of its sole diploid ($n=4$) in the Assam area. The geographic distribution of *I. prostratum*, is far wider, covering all parts of India and extending into Burma. Its success cannot be attributed to polyploidy alone, which is not even as high as that of *I. laxum*, but presumably to the onset of a favourable recombination through hybridisation of genetic types, adapted to diverse habitat conditions, such as those in the black cotton soils of Bundelkhand and the moist localities of the Carnatic. Relatively, therefore, it is not the step-wise reduction, but the origin of secondary basic numbers through amphiploidy, that has decided the areas of the Indian species of *Iseilema*. Their ultimate adaptation to specialised habitat conditions within the areas result from intraspecific genetic variation within the complex.

The Andropogoneae have attained a high development in the Indo-Malaysian region, with two zones of maximum species abundance, one in India (the Western Ghats, Bombay Deccan, Madhya Pradesh) and the other in Southern Indonesia (the Lesser Sunda Islands)¹. It is in this Indian zone of strong species preponderance, that *Iseilema* shows maximum cytological variability with unusually low chromosome numbers, providing a 'pool of chromosome sets', from which the higher chromosome numbers have been

derived mostly by amphiploidy and to some extent by auto-ploidy.

Such amphiploidy, involving any two parents with $n=3$, 4 or 5 and leading to the origin of secondary, tertiary and other complex basic numbers, explains the wide gamut of seemingly conflicting basic numbers, such as 5, 6, 7, 9, 10, 11, 12, 14, 15, 17, 18 and 19 encountered in the tribe Andropogoneae of the Indo-Malaysian region⁹. Perhaps it is the differential elimination of certain unsuitable types with $x=8$, 13 and 16 that has created gaps in this otherwise continuous series. It seems that all the basic numbers 3, 4 and 5 were present in the primitive flux of the Andropogoneae making the dibasic polyploidy and the evolution possible in the moist habitats of the Indo-Malaysian region, Hartley's ideas¹ being vindicated. The cytology of the Indian *Iseilema* thus puts the whole tribe Andropogoneae in its proper perspective both in regard to the long range chromosome reduction and the short range polyploidy, and takes us to the very root of Poaceae which, according to some cytologists, is characterised by a basic set of five chromosomes. In other words, the cytology of *Iseilema* illustrates the great series of chromosome changes which the tribe Andropogoneae has undergone since its early divergence from the original grass stock.

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Genetic relationships between brooding and brooded *Actinia tenebrosa*

SEA anemones of the genus *Actinia* seem to be dioecious and reproduce sexually, yet males, females, and individuals without gonads brood juveniles for much of the year¹⁻³. How this occurs is still not understood and knowledge on the subject is scant. Gillespie⁴ considered that eggs develop to ciliated blastulae and then directly into "a cylindrical form ... which develops tentacular buds". Chia and Rostron⁵ found no pre-planula larval stages in the coelenterons of hundreds of specimens they examined and postulated that most embryos must leave the mother as morulae or blastulae and later resettle in the coelenteric cavities of other adult *A. equina* where they metamorphose to post-Edwardsia juveniles. According to this hypothesis, juveniles contained in an adult should be derived from a number of different parents and would be expected to show a range of phenotypes characteristic of the population rather than the brooding adult. Cain⁶, however, found that in phenotypically varied populations of *A. equina*, young anemones invariably had the same colour as the adult from which they were released. He considered it likely that *A. equina* is a protandric hermaphrodite which mainly

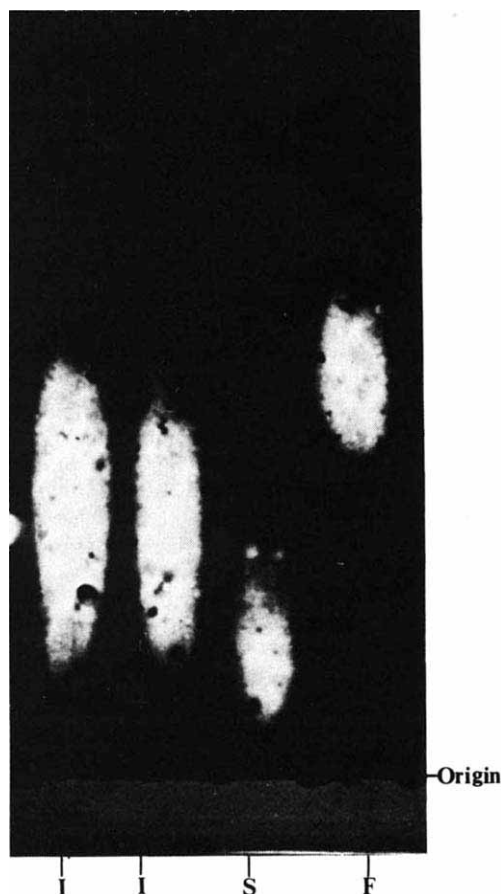


Fig. 1 Starch gel electrophoresis of *A. tenebrosa* catalase in 0.045 M Tris, 0.0008 M Na₂ EDTA, 0.025 M H₃ BO₃, pH 8.6 buffer. Catalase stain modified from Shaw and Prasad⁸. I, intermediate phenotype; F, fast phenotype; S, slow phenotype. Samples were frozen, ground in an equal volume of distilled water and centrifuged at 2,000g.

self-fertilises and retains larvae within the parent, even though Carlgren² thought this "hardly probable".

The intertidal anemone *A. tenebrosa* Farquhar is common on the shores of New Zealand and temperate regions of Australia. There is only one recorded colour phenotype⁶, so we examined *A. tenebrosa* for isozyme polymorphisms, similar to those known in *A. equina*⁷, to test Chia and Rostron's³ hypothesis.

Homogenised sea anemone tissue extracts were examined for eight enzymes⁸ using starch and gradient acrylamide ('Gradipore') electrophoresis. No traces of peroxidase or leucine aminopeptidase activity were found; there was no individual variation in soluble protein, esterase, acid phosphatase, alkaline phosphatase, alanyl aminopeptidase, or malate dehydrogenase. Three catalase phenotypes were found; fast, intermediate and slow (Fig. 1). From earlier studies of catalase isozyme polymorphisms^{9,10} we assumed that *Actinia* catalase is a tetrameric molecule; thus the fast and slow phenotypes were assumed to be homozygotes, whereas the intermediate phenotype was a heterozygote of 'fast' and 'slow' alleles. The intermediate phenotype of *Actinia* catalase should therefore contain five isozyme bands, but these were not distinguishable on our gels.

The sea anemones were collected from four localities near Adelaide, South Australia (Table 1). Phenotype frequencies varied between localities and in the Outer Harbour sample there was a highly significant excess of putative

Table 1 Catalase phenotype frequencies from different localities

Samples	Phenotypes			X test for Hardy-Weinberg frequencies	
	Slow	Intermediate	Fast		
Outer Harbour (Adelaide)	6	59	2	39.46	$P < 1\%$
Victor Harbour	0	3	9	—	—
Talisker Mines Beach (Cape Jervis)	0	5	5	—	—
Pennington Bay (Kangaroo Island)	16	5	0	0.38	—

heterozygotes, assuming a diploid diallelic system, with respect to Hardy-Weinberg frequencies. Some large brooded juveniles from the Outer Harbour sample provided sufficient catalase to give a staining reaction after electrophoresis. Phenotypes of 23 juveniles were determined (Table 2). Every juvenile examined had the same catalase phenotype as the host adult.

Our results are therefore consistent with those of Cain⁵, but we feel that the information available is insufficient and too contradictory to allow detailed speculation on the nature of the breeding system in *Actinia*. The correlation between phenotypes of adults and their brooded juveniles could indicate either asexual reproduction or apomictic parthenogenesis, but these systems would be inconsistent with previous observations of apparently functional male and female gonads. Furthermore, recent work raises an unlikely possibility that has not been dismissed: that a brooding adult may differentiate genotypes of brooded juveniles and reject those incompatible with its own.

Table 2 Phenotypes of juveniles extracted from adult *A. tenebrosa* in the Outer Harbour sample

Catalase phenotype of juveniles	Catalase phenotypes of nurse adults		
	Slow	Intermediate	Fast
Slow	5	0	0
Intermediate	0	15	0
Fast	0	0	3

Francis¹¹ showed that *Anthopleura elegantissima* distinguished between contacts of clonemates (of like genotype) and non-clonemates (of unlike genotypes), and reacted aggressively to the latter. *Actinia* also exhibits intraspecific conflicts¹², which may result in death of a juvenile involved in an adult-juvenile encounter (J. R. O., unpublished). Successfully brooded juveniles could therefore be only those genetically similar to the host, regardless of whether they resulted from cross-fertilisation of one or many matings. One approach to resolve this problem would be to breed adult *Actinia* in captivity for several years and compare fecundity of animals in solitary and group isolation with similar individuals and groups that were not isolated but had access to planulae from the sea.

Finally, we comment on Cain's assumption that *A. equina* is "so long lived". Substantial longevity data are available for only four species of sea anemones, all of which are for captive animals¹³⁻¹⁶. Only two of these records are for *A. equina*: one of Dalyell's specimens lived about 66 yr¹⁴ and another lived 14 yr¹³, but the lifespan of animals in such conditions may far exceed "ecological longevity" since a captive animal is usually protected from predators, hazards of the environment and scarcity of food¹⁷. Thus, although some actinians may have the potential to live many decades, it is possibly quite a rare occurrence in nature.

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Epidermal mucus and the reproduction of a crinoid echinoderm

THE unstalked crinoid *Comanthus japonica* spawns during the first part of October each year^{1,2}. For a study of gametogenesis³, specimens of *Comanthus* were collected periodically over 14 months. During these collections, one of us (J. G.) noticed that the animals became unusually slimy to the touch during the six to eight weeks that preceded spawning. This transient sliminess is correlated with an enlargement of an epidermal mucous cell population. The general position of the mucous cells is shown in Fig. 1, in which they are darkly stained. In both male and female genital pinnules, these cells occur mostly in the oral and lateral body wall. Each individual mucous cell is oval and contains a single nucleus. The cytoplasm stains with beta- to gamma-metachromasia in azure A. In addition, the cytoplasm stains strongly with alcian blue at pH 3, but fails to stain with periodic acid-Schiff. These histochemical criteria indicate an abundance of sulphated acid mucopolysaccharides (possibly mixed with non-sulphated acid mucopolysaccharides). It is not known if similar mucous cells occur in the arms and central mass of the crinoids, since these body regions were not preserved for histology.

In both sexes, the volume of the epidermal mucous cell population increases conspicuously in the weeks before spawning (Fig. 2). In the female (Fig. 2a), this increase is about ten times as great as in the male (Fig. 2b). The volume of the mucous cell population decreases markedly around the time of spawning in October in the female, but not in the male. Instead, the volume in the male declines gradually during the autumn and winter.

In both males and females, the population volume of the mucous cells increases abruptly in August. Therefore, the increase might be elicited in both sexes by the same environmental signal. The much greater increase in females could result from endocrinological differences between males and females; or there might simply be more mucous cells (or their precursors) in females. At present, it is not known if mucous cells are derived from or give rise to other cell types in adult crinoids. There is certainly no morphological evidence that the mucous cells are related to the so-called dorsal glands⁴ of the body wall.

The marked fluctuations in the size of the mucous cell populations around the time of spawning indicate that the

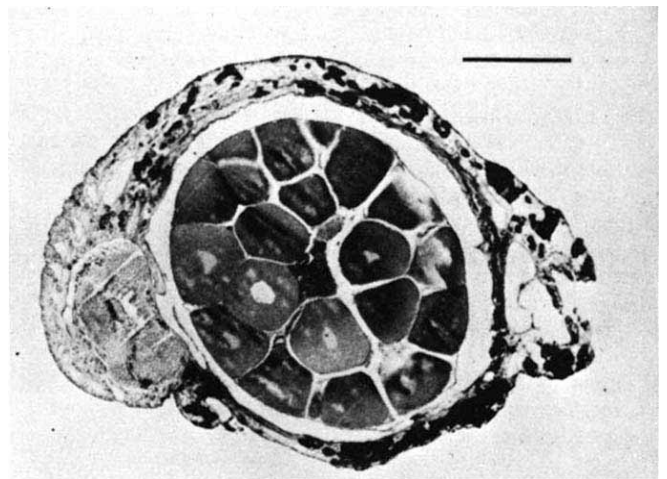
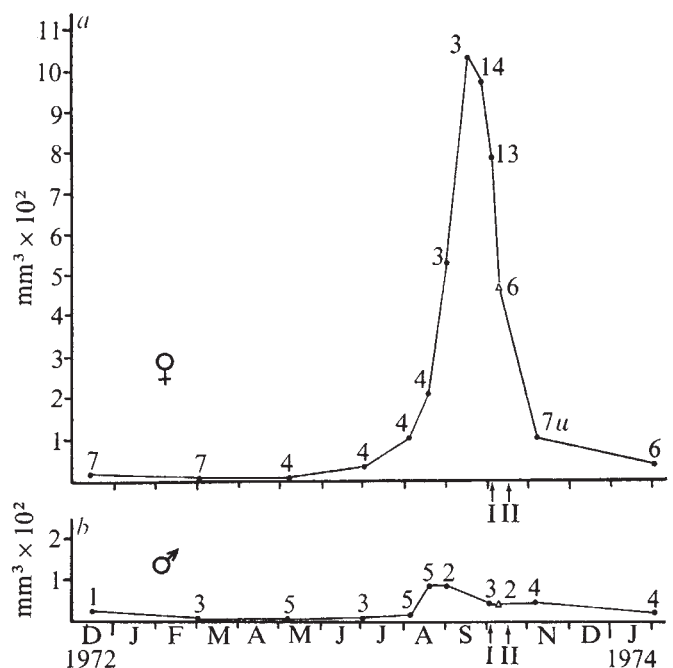


Fig. 1 Cross section of a genital pinnule from a female specimen of *Comanthus japonica* collected on September 15, 1973. The oral side with the food groove is at the right and the aboral side with the pinnular ossicle is at the left. The ovary, containing conspicuous oocytes, is separated from extragonadal portions of the pinnule by a genital coelom. Fixation was on the day of collection in seawater–Bouin's fluid. Staining³ was in alcian blue at pH 3. The epidermal mucous cells are the darkly stained objects in the oral and lateral body wall. The scale line represents 250 μ m.

epidermal mucus has some function in the reproductive biology of female crinoids. It is possible that the release of much epidermal mucus into the seawater by spawning females may act as a pheromone to stimulate epidemic spawning in nearby crinoids. Dan and Dan⁵ have shown, however, that such a pheromone is not necessary in all cases, since a male crinoid can spawn in isolation from spawning females (and *vice versa*).

Fig. 2 Volume fluctuations of epidermal mucous cell populations in females (a) and males (b) of *Comanthus japonica*. Volumes, given in $\text{mm}^3 \times 10^2$ per genital pinnule, were measured by the method of Holland *et al.*². The number of the individual females or males averaged for each data point is given above each collection date. For the collection of October 7, 1973, (Δ), only spawned animals were taken into account; the spawnings of the first and third weeks of October 1973 are marked, respectively, by arrows I and II. The November data point marked 'u' on the top graph is for animals which could not be sexed, most or all of which were probably females (see Holland *et al.*²).



Another, and more probable, function of the female's epidermal mucus may be to supply a temporary binding material for the newly released eggs. Dan and Dan⁵ discovered that the eggs of *Comanthus* are mixed with a viscous binding material which rises slowly through the seawater in a column above the spawning female. It was assumed⁵ that this material was produced within the ovary from dissolution of the jelly coats of eggs. But much (perhaps all) of the binding material may be supplied by the epidermal mucous cells. If there is a sudden secretion of a large part of the epidermal mucus at spawning, the epidermal mucous cells may well be responding to the same hormone(s) that presumably direct maturation, ovulation and spawning in this species.

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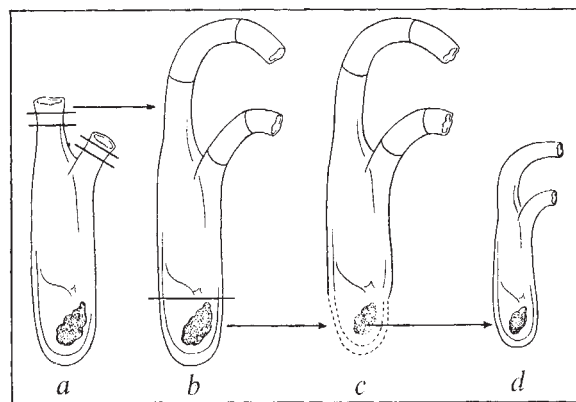


Fig. 1 Diagrammatic representation of the *Ciona* regeneration experiment purportedly done by Kammerer⁸. *a*, Distal halves of the incurrent and excurrent siphons of the animals were excised (cuts indicated by the solid line) and permitted to regenerate twice in succession, to produce animals with exceptionally long siphons as depicted in *b*. Regenerated siphons had a jointed or noded appearance. *b*, Gonad of the long-siphoned *Ciona* made to regenerate by cutting off (solid line) the basal part of the body which contains the gonad. *c*, Long-siphoned animals which had regenerated new basal parts and gonads (shown by dotted lines) were used to provide eggs and sperm for an F_1 generation. *d*, Young *Ciona* (F_1) resulting from this genetic cross were reported to have long siphons like the parents.

Siphon regeneration in *Ciona*

IN 1923 Paul Kammerer summarised research which he claimed demonstrated the inheritance of acquired characteristics. Since the success of Mendelian genetics had already made Kammerer's work suspect, a report of his lecture¹ was followed by a long and acrimonious debate. The Kammerer case thereby became a *cause célèbre* in the biology of that decade. The controversy has recently been resurrected in a popular book by Koestler² and has gained additional prominence from an internationally televised BBC documentary based on the book. This paper reports a failure to duplicate one of Kammerer's key experiments.

One of the examples cited by Kammerer purported to show that environmentally induced nuptial pads on the forelimbs of the male midwife toad, *Alytes obstetricans*, were inherited by the male offspring³. In 1926, however, this work was discredited by the finding that the nuptial pads on his one remaining *Alytes* specimen in the museum of the Vienna Institute for Experimental Biology were fraudulent⁴. Although Kammerer protested innocence of the forgery, the disgrace contributed to his suicide shortly afterwards⁵.

Koestler has staunchly defended Kammerer's scientific integrity², and on the basis of his presumed innocence in the matter of the midwife toad, and on the grounds that Kammerer's experiments were never properly repeated, he urges the scientific community to reinvestigate the work. Although there is ample reason to disagree with Kammerer's (and Koestler's) Lamarckian interpretations⁶, some of his experimental observations may nevertheless still be valid.

Kammerer himself believed that his experiments on siphon regeneration in the ascidian *Ciona intestinalis* L. were his best evidence for the inheritance of induced somatic changes^{7,8}, and Koestler particularly seeks a repetition of this work². The original experiment and its results are summarised in Fig. 1. Removal of parts of the incurrent and excurrent siphons (Fig. 1*a*) resulted in more elongated siphons being regenerated, even after a single excision. After a second removal of the newly regenerated siphons, still longer siphons were produced (Fig. 1*b*). The gonads were then made to regenerate by removing a basal segment of the animal containing the ovary and lower part of the intestine (Fig. 1*b* and *c*); Kammerer felt that the regenerating gonads would be more amenable to somatic influences. The F_1 progeny of these

gonad-regenerated animals were shown to have elongated siphons (Fig. 1*d*) like the parents.

In response to Koestler's appeal², I attempted to repeat this regeneration experiment during the summer of 1973. Adult *Ciona intestinalis* (8-12 cm long) were kept at Woods Hole in a tank in which the incoming fresh seawater was refrigerated to 12-15 °C. *Cionas* survive much better in cooler water than in the warmer laboratory sea tables. Before surgical amputations, the animals were anaesthetised with 0.01% tricaine methanesulphonate (MS 222; Sandoz Pharmaceuticals).

Two groups of 50 animals each had both siphons amputated: group 1 had the whole siphon removed at the base, and group 2 had the distal half of each siphon removed. Healing was quite rapid⁹ and new ocelli (reddish-orange photoreceptors¹⁰) appeared on the siphon edges within 4-6 d. Animals of both groups had completely regenerated their siphon lengths by 30-40 d, and showed no indication of siphon elongation nor any sign of a jointed or noded appearance of the siphons⁸.

A control group of 50 unoperated animals was kept in the same tank for comparison of relative siphon lengths. Group 1 were kept up to 60 d (38% survived compared with 68% of the control group) without any indication of excessive siphon growth. Likewise, the group 2 animals were kept for 53 d (56% surviving), also without siphon elongations beyond their normal proportions. Since siphon elongation was reported to occur even after a single amputation⁸, it seemed pointless to amputate the animals a second time.

These results agree with those of Fox¹¹, who attempted to repeat the *Ciona* experiments at the time of the original controversy. In addition to Kammerer's report, however, an earlier worker had also described siphon elongations in *Ciona* after a single amputation of the siphons¹². A suggestion¹¹ that these positive results could be caused by nutritional conditions which were known to produce elongated siphons in *Ciona*¹³, was flatly rejected by Kammerer¹⁴.

No attention seems to have been paid during the controversy² to the final phase of Kammerer's experiment in which he claimed to have obtained gonad regeneration. Gonad regeneration occurs in another ascidian, *Clavellina lepadiformis*¹⁵, and recent work shows that *Ciona* will regenerate a new ovary if the original is carefully removed through a small slit in the body wall¹⁶. Anyone actually trying the final operation depicted in Fig. 1*b* and *c*, however, could not fail to be im-

pressed by the improbability of the animals surviving such trauma. In fact, Hirschler¹⁷, in his extensive study of *Ciona* regeneration, found that animals could not survive this particular operation.

This point was investigated further by subjecting a third group of 50 normal animals to the gonad operation described by Kammerer⁸, and as depicted in Fig. 1b. All of the animals died between 3 and 6 d after the operation.

The failures of Fox¹¹ and myself to observe siphon elongation in regenerating *Ciona* suggest that elongation is not a commonplace occurrence and may indeed be caused only by special circumstances¹³. Kammerer's claims must therefore continue to be regarded with scepticism, especially as evidence presented here and elsewhere¹⁷ indicates that *Ciona* does not survive the gonad regeneration operation claimed to have been used by him.

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Temperature receptors on tarsi of the tsetse fly *Glossina morsitans* West

ELECTROPHYSIOLOGICAL techniques have shown that the responses of mechano- chemo- and olfactory receptors in insects are temperature-dependent^{1–3}, and several true thermoreceptors have been described^{4–8}. We have found thermosensitive cells on the tarsi of the tsetse fly that seem to be involved in probing behaviour. Electrophysiological recordings revealed spontaneously discharging cells in two hairs on the third, fourth and

Table 1 Probing responses of *G. morsitans*

	n	No. of flies settled on membrane	No. of flies probing	No. probing No. settled
Post-teneral males				
Accumulated controls	96	90	84	0.93
Antennae removed	43	?	2	
Pro. tarsi removed	61	57	47	0.82
Acetic acid on meso. tarsi	9	8	7	0.79
Acetic acid on pro. tarsi	33	26	3	0.12
Teneral males and females				
Accumulated controls	60	56	54	0.96
Pro. tarsi removed	45	41	26	0.63
Acetic acid on meso. tarsi	32	27	25	0.93
Acetic acid on Pro. tarsi	29	26	6	0.24

Post-teneral males were 1–3 weeks old and had been starved for 48 h. Teneral flies were starved for 24–48 h. Cages containing five to seven flies each were placed for 3 min on a 1-mm thick Agar/Parafilm membrane¹³ overlaying a feeding solution (10^{-3} M ATP in 0.15 M NaCl, warmed to 37 °C with pH adjusted to 7.0). Glacial acetic acid was applied after the inconclusive results of the tarsalectomy experiments. Electrophysiological experiments had shown that application of the acid to the tip of the hair gives an injury reaction: a burst of spikes followed by inactivity of the temperature receptor. To apply the glacial acetic acid, a glass tube (outer diameter about 1 mm), filled with the chemical, was placed over the leg of the fly. In this way evaporation of the acid, which may cause damage to other receptors, was minimised.

fifth prothoracic tarsomeres. These hairs are part of two ventrolateral rows of hairs probably similar to the receptors (re) described by Lewis for *Glossina palpalis*⁹. No spontaneously firing cells were found on the meso- or metathoracic legs. Detailed studies were made only on the fifth tarsomere hairs.

At room temperature, there were usually two cells firing in each hair. One gave large spikes (0.8–1.0 mV) at irregular intervals (on average 1–2 s⁻¹) and the other gave smaller spikes (0.3–0.6 mV) at a frequency of 20–40 s⁻¹. The large spikes gave occasional bursts, which often, though not always, coincided with a change in temperature. Air movement alone did not evoke the bursts, and some occurred without any detectable stimulus. Over a temperature range of 16–32 °C, the cell giving the small spikes showed all the characteristics of a cold receptor as defined by Hensel¹⁰ (Fig. 1). At other temperatures the amplitude of the spikes usually decreased to zero. Below 18 °C the decrease in amplitude was sometimes preceded by a sudden drop in frequency.

A sudden change in temperature caused a phasic increase

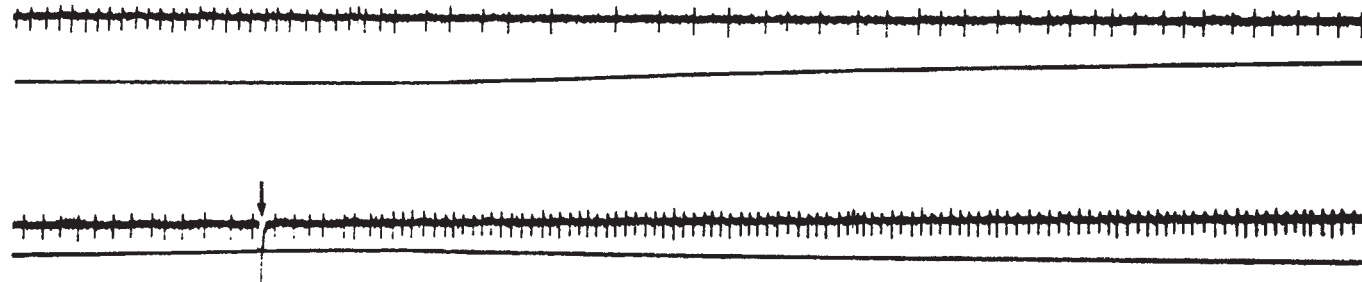


Fig. 1 Record of responses from a hair on the fifth tarsomere containing a cold receptor. The time bar represents 0.25 s. The fly's leg was cut off at the base of the tibia and mounted on a glass pipette filled with saline (0.15 M NaCl) which served as the indifferent electrode. Extracellular recordings were made from the tip of the hair by placing a glass pipette (outer diameter of tip 6 μ m) containing saline over the hair¹¹. A silver wire in the saline passed the signal to a W-P, VF-1 voltage follower, and the output was viewed on a Tektronix oscilloscope. Records were made with a Honeywell 1858 oscillographic recorder or with a Grass C-4 camera. Air at 2–50 °C was blown from a glass condenser over the leg, to obtain an ambient temperature range of 15–35 °C measured with a small (diameter 0.095 inch) epoxy coated thermistor mounted approximately 5 mm from the tip of the hair. The top trace represents the response of the cold receptor to an application of a warm airstream, while the third trace represents the response of the same cell to removal of the warm airstream (arrow and electrical artefact). The recording is continuous. The line under each electrophysiological trace represents the response of the thermistor. The response of the thermistor is not necessarily a true indication of the rate or magnitude of the temperature change at the surface of the receptor. With longer applications than shown here, however, it was possible to get an approximation of the magnitude of the temperature change when the thermistor had stabilised (see Fig. 2a).

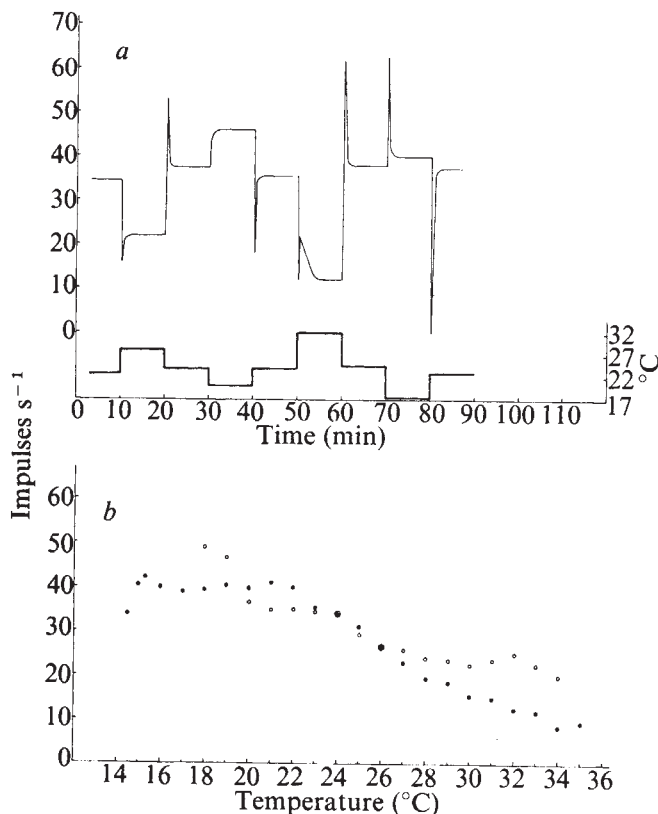


Fig. 2 *a*, Responses of a single cold receptor (top graph) to rapid changes in temperature followed by steady temperatures (bottom graph). The air in the condensor was held at various temperatures (above or below room temperature) and then applied suddenly to the preparation (see Fig. 1). The preparation was held subsequently at each new temperature for 10 min before being returned suddenly to room temperature for 10 min. An approximation of the magnitude of the temperature change was obtained in each case when the thermistor equilibrated. The changes are plotted as instantaneous, and must have been very fast, at the receptor level, judging from its response. *b*, Response of a single cold receptor to slow changes in temperature. The temperature was allowed to increase from about 15 °C to over 34 °C, and the response of the cell was monitored at regular intervals (●). After 169 min, the temperature was decreased, and cell response was again plotted (○) for 64 min.

(cooling) or decrease (warming) of spike frequency, of a size determined both by the extent of the temperature change and by the initial steady temperature (Fig. 2*a*). Frequencies up to 80 spikes per s were recorded in response to a rapid decrease in temperature. When temperature changed slowly (1 °C per 3–5 min), from approximately 21 to 34 °C, the firing frequency of the cell followed almost linearly ($Q_{10}=0.4-0.7$) (Fig. 2*b*). Above 40 spikes per s tonic reactions of the cell became unpredictable.

To test the possibility that the temperature receptor may also respond to chemicals, NaCl (1.0 M), urea (0.1 M) and LiCl (0.3 M) were each applied to the hair. In each case, the temperature receptor functioned normally.

Of what value are these thermosensitive cells to the tsetse fly? Electrophysiological identification of such receptors gives no clue to any influence they may have on the animal's behaviour. Dethier¹² showed that heat is the major stimulus that induces probing, and that the receptors detecting the heat source are on the antennae, rather than on the palpi. Although he mentioned temperature sensitivity of the legs, only chemical responses were studied in detail. When we removed the prothoracic legs, results were variable but there tended to be a reduction in the number of flies which probed. This was easier to demonstrate in teneral flies (newly emerged flies before their first meal) than in post-teneral flies. Treatment of the prothoracic legs with glacial acetic acid eliminated the

probing response in most teneral and post-teneral flies (Table 1). In a control group, with mesothoracic legs treated with glacial acetic acid, the probing response was not altered significantly. We conclude that, in its probing response, the tsetse fly uses a secondary input from the temperature receptors on the tarsi, in addition to the primary input from the receptors on the antennae. Why acetic acid treatment disrupted probing more effectively than removal of the prothoracic legs is unknown.

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Passive immunisation of marmoset monkeys against neoplasia induced by a herpesvirus

THE malignant lymphoma induced by herpesvirus saimiri (HVS) in non-human primates¹ provides a model system for the study of the prevention of neoplasia in man induced by suspected oncogenic herpesviruses². We have reported that cotton topped (CT) marmoset monkeys (*Saguinus oedipus*) actively immunised with a killed HVS vaccine were resistant to the inoculation of the oncogenic HVS while the non-vaccinated control monkeys died of malignant lymphoma³. In certain virus infections (for example, measles, infectious hepatitis, German measles) passive immunisation by administration of specific serum antibodies during the incubation period may result in prevention or modification of the clinical disease. This paper describes the prevention of herpesvirus induced malignant lymphoma in monkeys by passive immunisation.

For all experiments described HVS isolate S.295C¹ was used. The virus was cultivated on owl monkey kidney (OMK) cells⁴ and stored at 4 °C as stock virus. For the passive immunisation of CT marmosets whole serum passed through Millipore membrane filters (450 nm) was used. The monkeys received the immune serum by intramuscular inoculation in the lower extremities and were challenged 24 h later by intramuscular inoculation of HVS in an upper extremity. Serial tenfold dilutions of filtered (450 nm) stock virus were prepared for challenge. Aliquots (1 ml) of each dilution were injected into passively immunised monkeys and also into non-immunised control monkeys. The infectivity titre (TCID₅₀) of the virus dilutions was determined in parallel in OMK cells.

CT marmosets with HVS induced malignant lymphoma developed high titres of specific serum antibodies against HVS⁵. In the first experiment immune serum obtained from tumour-bearing CT marmosets was used. One of the two immune sera tested was obtained from a CT marmoset that had received 2×10^6 freshly prepared tumour cells derived from an animal of the same species with HVS induced

Table 1 Challenge of CT marmoset monkeys with live HVS

	Dilution of HVS stock virus	Titre of inoculum LD ₅₀ * TCID ₅₀ †		Survival time after inoculation of HVS (d)	Infectivity titre of whole blood (TCID ₅₀ ml ⁻¹)‡
Passively immunised monkeys	10 ⁻¹	3,162	1,000	134§	10 ^{5.0}
	10 ⁻³	32	10	73§	10 ^{5.5}
	10 ⁻³	32	10	79§	10 ^{5.5}
	10 ⁻³	32	10	346¶	< 10 ^{0.0}
Non-immunised control monkeys	10 ⁻¹	3,162	1,000	33§	10 ^{5.3}
	10 ⁻³	32	10	23§	10 ^{2.5}
	10 ⁻³	32	10	45§	10 ^{6.5}
	10 ⁻³	32	10	47§	10 ^{6.5}

The monkeys were passively immunised with serum obtained from CT marmosets which had died of malignant lymphoma.

*Determined in experiment No. 2 (Table 2).

†Determined in OMK cells.

‡Determined in OMK cells at the given survival time.

§Monkey died of malignant lymphoma.

¶Monkey is clinically well.

malignant lymphoma. The recipient CT marmoset died of malignant lymphoma 36 d after the cell transplantation. The serum was taken from this monkey at the time of death. The neutralisation index of this serum determined in OMK cells according to standard techniques⁶ was 3.0. The serum (6 ml) was inoculated in a CT marmoset. This monkey was challenged with the 10⁻¹ dilution of the stock virus. The TCID₅₀ of the 10⁻¹ dilution was 1,000 (Table 1) and corresponded to 3,162 LD₅₀ calculated according to the method of Reed and Muench (Table 2). The immunised monkey showed delayed tumour development and survived 134 d whereas the non-immunised control monkey died 33 d after inoculation of malignant lymphoma (Table 1). The second immune serum was obtained from four CT marmosets that had been inoculated with 10 TCID₅₀ of cell free HVS stock virus. All four monkeys had died of malignant lymphoma 34–42 d after infection. The neutralisation index of the pooled serum from these monkeys was 2.8. Three CT marmosets each received 6 ml from this serum pool. These three monkeys were challenged with the 10⁻³ dilution of the stock virus which contained 10 TCID₅₀ of HVS corresponding to 32 LD₅₀. While the three non-immunised control monkeys died of malignant lymphoma 23–45 d after inoculation, two of the passively immunised monkeys died of the

tumour as late as 73 and 79 d after inoculation and the third of the passively immunised monkeys survived without any sign of a clinical disease or infection with HVS as monitored by cocultivation of its peripheral blood with OMK cells⁷. This animal has now been under observation for 346 d (Table 1).

For the second experiment the serum for the passive immunisation was obtained from CT marmosets actively immunised with a killed HVS vaccine as described previously⁸. None of these monkeys showed signs of a HVS infection⁴. Blood was drawn from 24 vaccinated CT marmosets at different time intervals after the immunisation with the HVS vaccine and the sera were pooled. The neutralisation index of the serum pool was 3.5 and the titre of the complement fixing (CF) antibodies was 1:32. The CF titres were determined according to standard techniques⁹ using a HVS antiserum from an owl monkey⁴. Six CT marmosets each received 6 ml of this serum. The titres of neutralising serum antibodies in the peripheral blood of these monkeys were determined 24 h later, before challenge. At this time the neutralisation indices ranged from 1.8 to 2.0. After 103 d they had dropped to nearly zero. These monkeys were challenged with the 10⁻¹ and 10⁻³ dilutions of the stock virus. Both the passively immunised monkeys that had been

Table 2 Challenge of CT marmoset monkeys with 10 and 1,000 TCID₅₀ of live HVS

	Dilution of HVS stock virus	Titre of inoculum LD ₅₀ TCID ₅₀ *		Survival time after inoculation of HVS (d)	Infectivity titre of whole blood (TCID ₅₀ ml ⁻¹)†
Passively immunised monkeys	10 ⁻¹	3,162	1,000	63‡	10 ^{5.5}
	10 ⁻¹	3,162	1,000	74‡	10 ^{5.5}
	10 ⁻³	32	10	204§	< 10 ^{0.0}
	10 ⁻³	32	10	204§	< 10 ^{0.0}
	10 ⁻³	32	10	204§	< 10 ^{0.0}
	10 ⁻³	32	10	204§	< 10 ^{0.0}
	10 ⁻³	32	10	204§	< 10 ^{0.0}
	10 ⁻¹	10 ^{3.5}	10 ^{3.0}	31‡	10 ^{4.8}
	10 ⁻¹	10 ^{3.5}	10 ^{3.0}	34‡	10 ^{5.0}
	10 ⁻³	32	10	42‡	10 ^{5.5}
Non-immunised control monkeys	10 ⁻³	32	10	44‡	10 ^{5.5}
	10 ⁻³	32	10	45‡	10 ^{5.5}
	10 ⁻³	32	10	52‡	10 ^{5.5}
	10 ⁻⁴	3.2	1	36‡	10 ^{4.8}
	10 ⁻⁴	3.2	1	44‡	10 ^{5.5}
	10 ⁻⁴	3.2	1	52‡	10 ^{6.3}
	10 ⁻⁴	3.2	1	126‡	10 ^{4.5}
	10 ⁻⁵	< 1	< 1	204§	< 10 ^{0.0}
	10 ⁻⁵	< 1	< 1	204§	< 10 ^{0.0}
	10 ⁻⁵	< 1	< 1	204§	< 10 ^{0.0}
	10 ⁻⁵	< 1	< 1	204§	< 10 ^{0.0}
	10 ⁻⁵	< 1	< 1	204§	< 10 ^{0.0}
	10 ⁻⁵	< 1	< 1	204§	< 10 ^{0.0}
	10 ⁻⁵	< 1	< 1	204§	< 10 ^{0.0}

The monkeys were passively immunised with serum obtained from CT marmosets actively immunised with a killed HVS vaccine.

*Determined in OMK cells.

†Determined in OMK cells at the give observation time.

‡Monkey died of malignant lymphoma.

§Monkey is clinically well.

challenged with the 10^{-1} dilution died of malignant lymphoma. The tumours appeared only after a delay, however, and the monkeys died 63 and 74 d after the challenge whereas the non-immunised control monkeys died 28 and 34 d after inoculation (Table 2). All of the four passively immunised monkeys that received the 10^{-3} dilution survived without any sign of disease or virus replication and have now been under observation for 204 d. In contrast, all of the non-immunised monkeys that received the 10^{-3} dilution died 23–47 d after inoculation of malignant lymphoma and even all four of those control monkeys that received the 10^{-4} dilution developed a tumour (Table 2).

These experiments demonstrate clearly that non-human primates can be protected against HVS induced malignant lymphoma by passive immunisation. The passively immunised CT marmoset monkeys were resistant to small dosages of cell-free HVS during an observation period of 204 and 346 d whereas the non-immunised control monkeys died of malignant lymphoma. Challenge with doses higher than 32 LD₅₀ of HVS regularly induced a tumour in the passively immunised CT marmosets. The development of malignant lymphoma, however, was considerably delayed in these monkeys as compared with the non-immunised control monkeys. The serum obtained from tumour-bearing CT marmosets protected another CT marmoset against a tumour of the same kind. The fact that the humoral antibodies were not able to stop the growth of an established tumour in the donor of the serum indicates that a passive immunisation may only protect if carried out before the intracellular establishment of the infection. Humoral antibodies do not protect against the cellular transmission of herpesviruses⁹ and antibodies against herpes simplex virus types 1 and 2 provide little protection against recurrent disease in man¹⁰. The active immunisation of CT marmosets with a killed HVS vaccine⁸ protected against malignant lymphoma induced by the inoculation of cell free HVS³. The fact that the protection could be transmitted with the serum of the vaccinated monkeys indicates that humoral antibodies are important in resistance to HVS in those experiments. In addition, the results obtained with passive immunisation against HVS support the concept that HVS is the aetiological agent for the tumours.

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malignant murine cells may be more sensitive to increased temperature than their normal counterparts^{1,2}; temperatures in the range 39–43 °C sensitise cells, and particularly radio-resistant hypoxic cells, to X rays^{3–6} and to chemotherapeutic agents^{7–9}; and humans can stand prolonged exposures to temperatures up to 43 °C, provided adequate supportive precautions are taken^{10,11}.

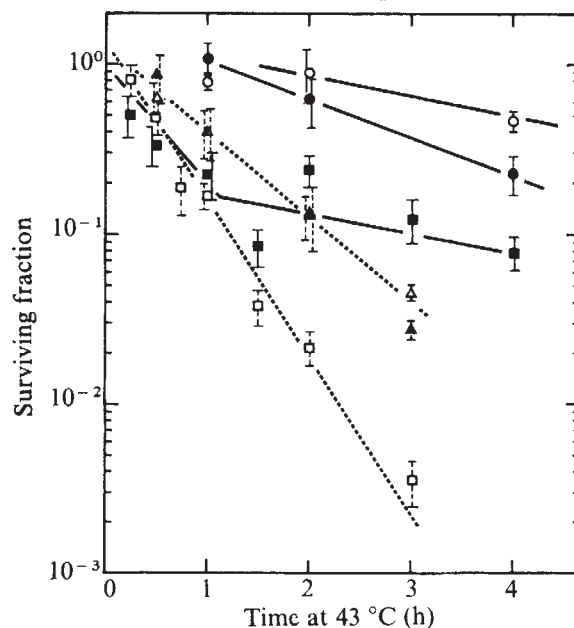
Surprisingly, the literature contains essentially no quantitative data on the thermal response of normal human cells; *a fortiori* there are no data on comparative response of normal cells and similar cells shown to be malignant by implantation into immunodeficient animal hosts. We have therefore initiated such an investigation using the embryonic lung fibroblasts WI-38¹² and SV-40 transformed WI-38-VA13/2RA¹³. Our findings indicate that, indeed, the transformed cells are consistently more sensitive to 43 °C exposures. Furthermore, we show that the kinetics after heat shock of the two cell populations are different, a finding which may have application in sequential heat-X ray or heat-chemotherapy.

Both cell strains were tested for tumorigenicity by injecting 2×10^5 – 2×10^6 cells subcutaneously into the flanks of homozygotic *nu/nu* mice developed on an NIH background at Stanford Medical Center and maintained under aseptic conditions. One of five mice injected with WI-38-VA13/2RA cells developed a visible nodule within 10 d of injection. None of the three mice injected with WI-38 cells developed nodules. These results are similar to those published elsewhere¹⁴ for other malignant human cells. Furthermore, essentially similar results were shown after implantation of cells of a similar origin as WI-38 and WI-38-VA13 cells into terminal cancer patients^{15,16}.

The cells were plated in 60 mm plastic Petri dishes at 1×10^5 – 2×10^5 cells per dish in 5 ml of Eagle's MEM supplemented with 15% foetal calf serum medium. Cells in exponential growth were used on either day 1 or day 2 after plating. The population doubling time in exponential growth varied from 22 to 32 h. Cells reached a confluent monolayer in 9 to 11 d after plating with no change of medium. These plateau-phase cells were used either with no change of medium or with fresh medium applied on the day before heating and again on the day of heating.

Cells were heated by placing replicate dishes in a rack which

Fig. 1 Survival of exponentially growing cells (■, WI-38 and □, 2RA) and plateau-phase cells (●, WI-38, fed; ○, WI-38, unfed; ▲, fed; △, 2RA, unfed) exposed for various times at 43 °C. Unfed cells had no medium change since planting. Fed cells had a medium exchange on the day before and the day of the heat treatment. Error bars represent 1 s.e.m.



Differential heat response of normal and transformed human cells in tissue culture

MUCH interest has been rekindled in the possible use of hyperthermia either alone or as an adjunct to more conventional treatments in the management of malignant disease. There are several reasons for this renewed interest: data indicate that

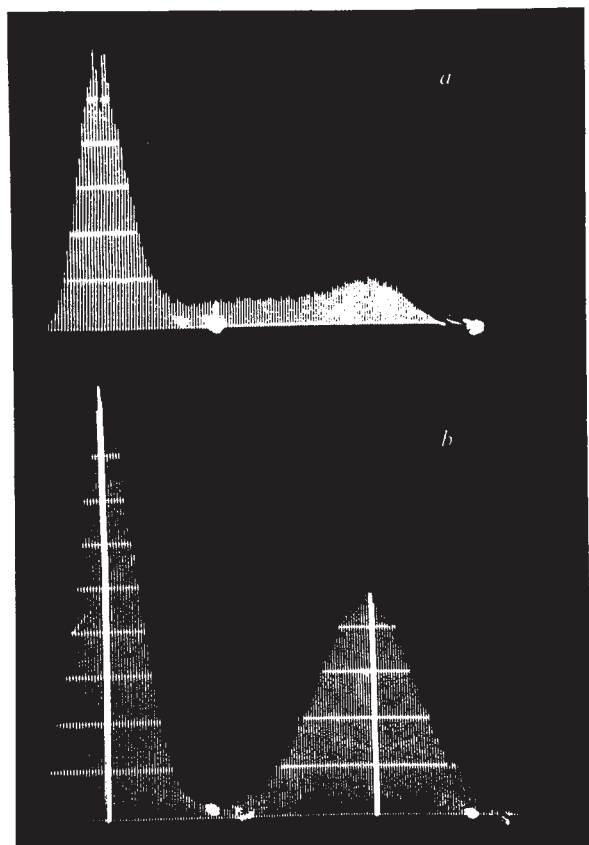


Fig. 2 DNA distributions of WI-38 cells. *a*, Normal distribution of exponentially growing cells; *b*, distribution of cells 24 h after heating at 43 °C for 4 h.

allowed the cells and growth medium to be immersed in a 43 °C water bath. The water bath was inside an incubator and a mixture of air and 5% CO₂ was bubbled through the water constantly. Water temperature was controlled to ± 0.1 °C by a proportional controller (Versatherm Model 2156, Cole-Parmer Co.).

For survival experiments, cells were detached after heat treatment using 0.05% trypsin and replanted at the appropriate dilution in 60-mm or 100-mm plastic Petri dishes for cloning at 37 °C. Cloning efficiencies varied from 5% to 35%. Survival results, illustrated in Fig. 1, show that generally the transformed cells exhibited a lower survival than the normal cells when given the same exposure to 43 °C in the same conditions of cell growth, confirming observations with murine cells^{1,2}. Fresh medium seems to have had a slight sensitising effect on the normal cells. Also the exponentially growing cells survived less well than plateau-phase cells. This result is contrary to that found with one other mammalian cell line¹⁷.

To determine the effect on cell population kinetics during and after exposure to 43 °C, we studied the changes in the DNA distribution of the cell populations after heating. Several dishes of exponentially growing cells were heated at 43 °C for a fixed time. At various times up to 6 d after heat treatment, replicate dishes were trypsinised and the cells were fixed and stained with ethidium bromide¹⁷. The cell suspension was then passed through a commercially available fluorescence flow system (Cytofluorograph 4801, BioPhysics Systems, Inc.); output signals from which were accumulated on a multichannel analyser. Since the uptake of dye per cell is directly proportional to DNA content¹⁸, the analyser output is a representation of the DNA distribution of the cell population. Typical distributions are shown in Figs 2 and 3, which also show typical shifts in the distributions after heating. Cells in G₁ appear in the first peak

while cells in G₂-M, with a double DNA complement, appear in the second peak. S-phase cells fall in between the two peaks.

A sample containing equal numbers of each cell strain was also analysed to determine the relative DNA complements of the two strains. This analysis shows that the DNA complement of the transformed cells was almost twice that of the normal diploid cells, confirming that tetraploid cells dominate the population in the transformed strain¹⁹.

Changes in the DNA distributions with time after heating at 43 °C are shown in Fig. 4. The points represent data from two or three experiments. The heating times were chosen to give about the same fractional survival in both cell populations

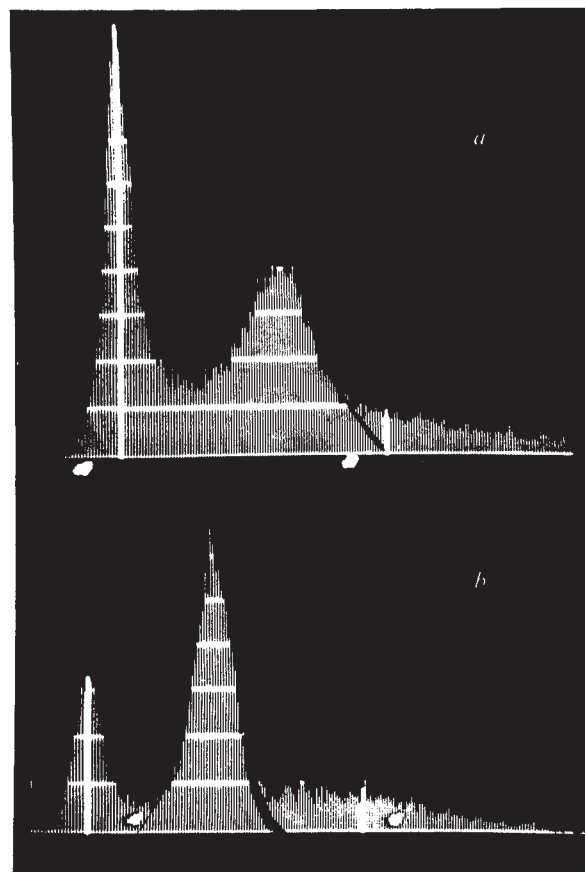


Fig. 3 DNA distributions of WI-38-VA13/2RA cells. *a*, Normal distribution of exponentially growing cells; *b*, distribution of cells 46 h after heating at 43 °C for 90 min.

(0.05). Accumulation of S-phase cells while the cells were at 43 °C together with a depletion of cells in G₂-M may indicate a heat-caused block in the progress from S to G₂ in the transformed cells. Depletion of G₁ also indicates a mitotic block during heating. After the return to 37 °C there was a steady increase in the G₂-M population in both cell strains for 20–60 h, with a corresponding decrease in G₁ and S populations. During the first 20 h this accumulation was probably the result of a continuing mitotic block for all cells. The populations were dominated by non-viable cells during the first few days after heating; the continuing accumulation in G₂-M is thought to result from an accumulation of cells in late G₂ or mitosis before cell death. Beyond 60 h viable cells have proliferated sufficiently to influence the DNA distributions and these gradually return to normal.

Assuming that the WI-38-VA13/2RA cells model the malignant cells of a human tumour whereas the WI-38 cells model the associated normal tissue, there seem to be inherent

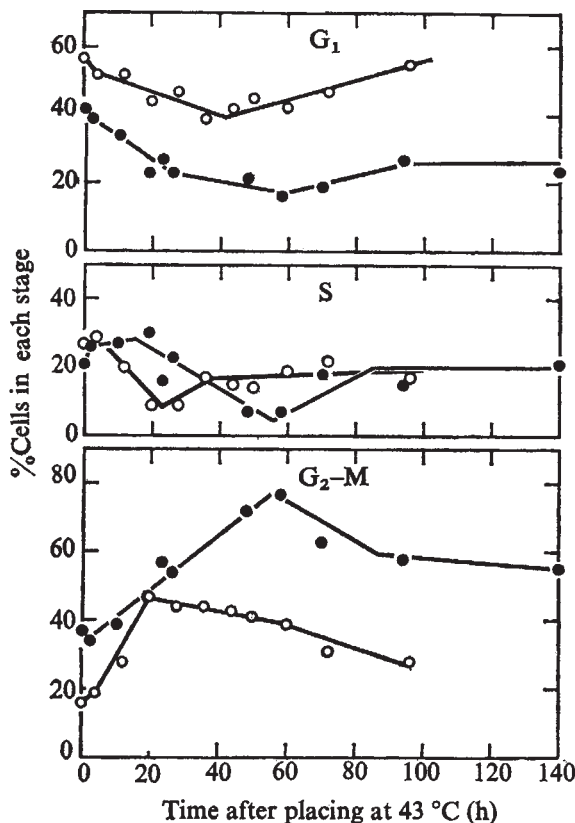


Fig. 4 Variation with time after heating of the DNA distribution for WI-38 cells heated at 43°C for 4 h (○) and WI-38-VA13/2RA cells heated at 43°C for 90 min (●).

differences in the hyperthermic response which may be exploited in cancer treatment. Not only are the transformed cells more readily destroyed by hyperthermia, but the differential cell kinetics that follow heating suggest that fractionated treatments involving either hyperthermia alone or in combination with radiotherapy or chemotherapy could further improve the therapeutic ratio.

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Organ specificity of blood-borne tumour metastasis determined by cell adhesion?

ONE of the most clinically troublesome aspects of cancer is metastasis, the spread of tumour cells from primary to distant, multiple secondary sites¹⁻³. Metastasis initially occurs through tissue invasion, followed by transportation of tumour cells through the circulatory system, lymphatics or coelomic cavities^{1,2}. In blood-borne metastasis free tumour cells or cell aggregates are carried into various organs and tissues where they stop in the first capillary bed encountered; however, most of these arrested tumour cells break loose, recirculate and stop in other organs^{4,5}. In many tumour systems the location and extent of established secondary tumour colonies are non-random, suggesting that unique cell properties are important in tumour arrest and metastasis^{3,6}. In support of this hypothesis Fidler⁷ was able to select from a murine B16 melanoma line variants that showed enhanced metastatic behaviour by repeated cycling of tumour cells from lung colonies to tissue culture, administering the tumour cells intravenously on each cycle. We report here the organ adhesive specificity of four B16 variant cell lines selected for enhanced lung metastasis.

B16 variant melanoma lines with increasing ability to form lung metastases from subcutaneous or intravenous administration were obtained from Dr I. J. Fidler (University of Pennsylvania). These B16 lines are designated F1, F5, F10 and F13 based on the number of *in vivo* selections. The metastatic properties of these B16 variants are stable and not dependent on cell growth conditions⁷. Melanoma variants were grown *in vitro* in Dulbecco's modified Eagle's MEM⁸ (DMEM) supplemented with 10% foetal calf serum, glutamine and non-essential amino acids. Cells were collected with EDTA as described previously⁹. The variant lines were also grown *in vivo* by intraperitoneal implantation in syngeneic C57BL/6J mice. After 2 weeks cells were collected from the peritoneal cavities and gently dispersed in DMEM through 200-mesh stainless steel filters. Contaminating phagocytic cells averaged less than 5% and viability assessed by dye exclusion averaged greater than 96%.

To assay organ cell adhesive properties single cell suspensions of lung, liver, kidney, brain and spleen were obtained from naive, syngeneic animals as follows. Organs were removed from 6-12 adult mice, pooled according to organ type and cut into 2-4-mm pieces in 10 ml of cold DMEM without serum and washed in DMEM. All steps were performed at 4°C. Dispersion of organs into single cells was accomplished by gently teasing the tissue pieces over the stainless steel filter with a Teflon policeman. Cell suspensions were rinsed by centrifugation (800g, 10 min) twice with 15 ml volumes of 0.05 M sodium phosphate-buffered saline (PBS) pH 7.4. Single, viable organ cells were isolated from cell debris, erythrocytes, platelets and cell aggregates by the two-step isopycnic-rate zonal gradient technique of Perper *et al.*¹⁰. Isopycnic centrifugation in Ficoll-Hypaque gradient yielded viable single cells with some membrane contamination. Subsequent centrifugation in discontinuous sucrose density gradients (5-20-30% w/v sucrose in PBS) separated erythrocytes, platelets and membrane debris (5% layer) and some minor cell-aggregates (30% layer) from single organ cells (20% layer). Final organ cell suspensions were rinsed free of sucrose by centrifugation in PBS and contained less than 5% erythrocytes by conventional

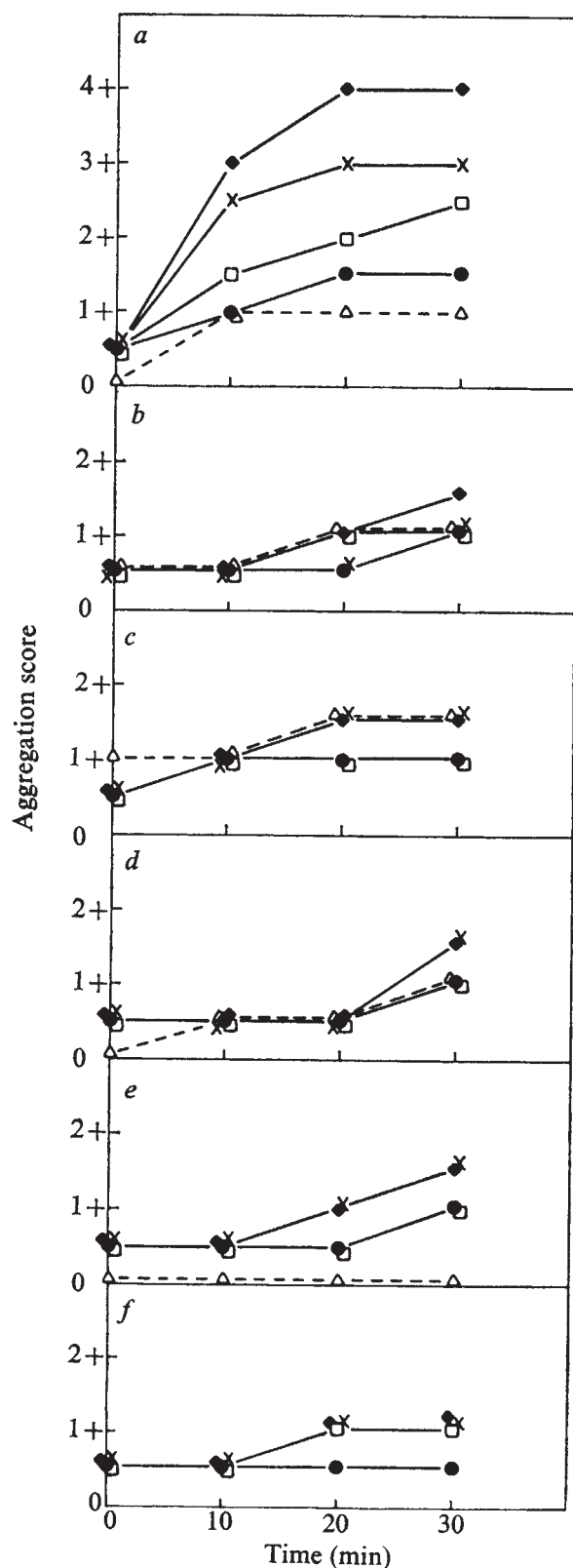


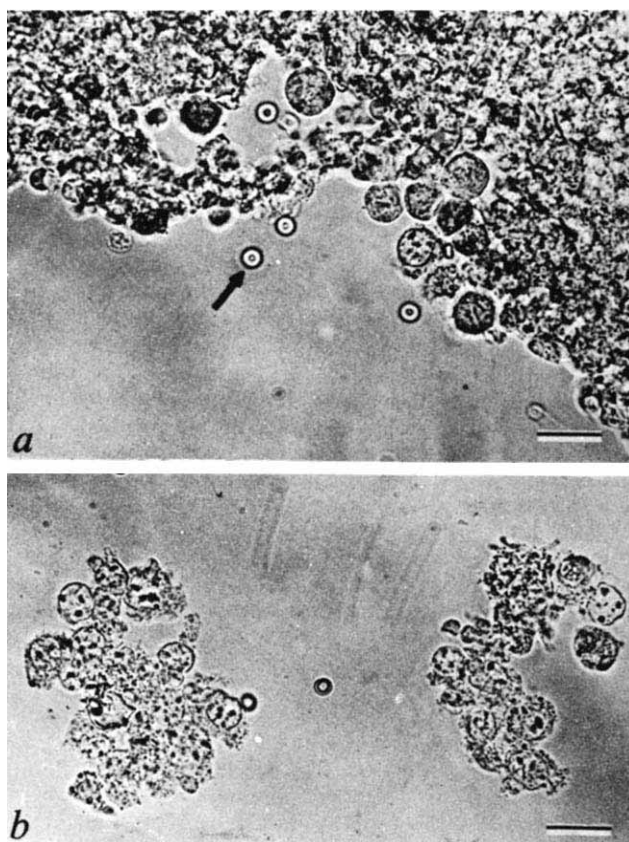
Fig. 1 B16 tumour cell aggregations with murine C57BL/6J organ cells. *a* Lung; *b* liver; *c* spleen; *d* kidney organ cell suspensions; *e* erythrocytes and *f* tumour cells alone. Extent of aggregation is scored from 0 (no aggregation) to 4+ (complete aggregation) (see text for details on the assay). These results were confirmed in three separate experiments using coded triplicate samples for each point. B16 melanoma cells were used at $1 \times 10^6 \text{ ml}^{-1}$. $\bullet$, F1; $\square$, F5; $\times$, F10; $\blacklozenge$, F13 and $\triangle$, organ cells. Lung, liver, spleen and kidney were used at 3×10^6 – $5 \times 10^6 \text{ ml}^{-1}$ whereas the erythrocyte concentration was $1 \times 10^7 \text{ ml}^{-1}$.

Wright's stain. Viability was greater than 97% as judged by dye exclusion, and contamination by phagocytic cells averaged less than 7%.

The aggregation assay was performed in 0.4 ml of medium containing 2% foetal calf serum using Linbro FP54 trays. Cells were dispensed into each well on the basis of their approximate surface area (see legend to Fig. 1), and the trays were rotated at 2 Hz on a rotary platform^{9,11,12} for various times at 37 °C. Aggregation was scored as before⁹ from 0 (no aggregation; 90–100% single cells), 1+ (scant aggregation; 60–90% single cells), 2+ (moderate aggregation; 30–60% single cells), 3+ (strong aggregation; 5–30% single cells) to 4+ (complete aggregation; 0–5% single cells). The reliability of this assay has been monitored and confirmed by electronic particle counting techniques¹² and adherence of ^{125}I -5-iodo-2'-deoxyuridine-labelled cells to cell monolayers J. L. W. and G. L. N., unpublished).

When melanoma variant cell lines are suspended with partially purified organ cells, heterotypic aggregation occurs¹³. This aggregation is specific and is related to the preferred organ site for implantation. The tumour lines selected for more lung metastases showed greater organ specific aggregation: lung > liver, kidney, spleen, erythrocytes (Fig. 1). With suspended lung cells the extent of aggregation paralleled the number of metastatic selections that the lines had undergone *in vivo*: F13 > F10 > F5 > F1 (Fig. 1a). There was less aggregation with non-target organ cells, with little indication of selective aggregation by the more metastatic lines (Figs 1b–e). B16 tumour cells also homotypically (self) aggregate (J. L. W. and G. L. N., to be published). There was significantly less homotypic than heterotypic aggregation with lung cells (Fig. 1f), but the

Fig. 2 Heterotypic cell aggregates formed with B16 variants and organ cells. *a*, B16-F13 variant aggregated with lung cell suspension for 20 min at 37 °C results in complete aggregation and the formation of one large mixed cell aggregate (4+ aggregation); *b*, B16-F5 variant aggregated with lung cells for 20 min at 37 °C (2+ aggregation). Arrow indicates unaggregated erythrocyte. $\times 420$; bar represents 10 μm .



difference was small or non-existent in homotypic and non-lung heterotypic aggregation experiments (Fig. 1b-e and our unpublished results). Microscopic examination of wells containing 4+ aggregates of lung and F13 cells revealed practically no free cells, indicating complete aggregation (Fig. 2a). Heterotypic aggregates formed with lung cells and B16 lines or with other organ cell suspensions were heterogeneous in cell composition (Fig. 2b). The existence of mixed aggregates of tumour and organ cells was confirmed by double labelling techniques (work unpublished). Soon after initiating the assay (10 min) there was little evidence of nonspecific cell aggregation (Fig. 1e-f).

The preferential adhesion to lung cells of the increasingly metastatic B16 variant lines seems to be paralleled by tumour cell behaviour *in vivo*. Injection of ¹²⁵I-labelled cells of the B16 variant into the tail vein of syngeneic mice results in 100% lung arrest of the B16-F11 and -F6 lines and approximately 80% lung arrest of the F1 line within 2 min. By 4 h 90, 80 and 55% and after 3 d 47, 35 and 2% of lines F11, F6 and F1, respectively remain in the lungs¹⁴. These results parallel closely the number of resulting lung tumour colonies visible within 3-4 weeks in the animals. Subcutaneous implantation of B16 metastatic variant cells in syngeneic hosts followed by removal of the primary subcutaneous tumours 1 to 2 weeks later results in spontaneous lung metastases within 6-8 weeks, the number formed being proportional to the number of *in vivo* selections F10>F5>F1 (ref. 14). These results indicate that initial cell-cell recognition during tumour cell arrest in specific organs may be one of the most important steps in the spread of blood-borne tumours.

Recent observations suggest that the success of tumour implantation and subsequent metastatic growth are determined by unique cell surface properties. Treatment of tumour cell surfaces with trypsin, neuraminidase¹⁵⁻¹⁷, heparin^{18,19} or dextran²⁰ before intravenous introduction modifies arrest and tumour distribution. Surface interaction of blood-borne tumour cells with lymphocytes¹⁴ and platelets²¹ can also affect the fate of circulating tumour emboli. Using B16 metastatic variants, Bosmann *et al.*²² reported differences in surface proteases, glycosidases, glycosyltransferases and electrophoretic mobilities between low and high metastatic lines. These studies suggest the importance of cell surface properties in determining the *in vivo* fate of tumour cells.

Our results argue against an entirely nonspecific trapping mechanism for circulatory tumour cell arrest^{1,2} and suggest that cellular recognition is important in determining the organ specificity of metastatic tumour spread. Since highly metastatic B16 tumour cells cause virtually complete aggregation of suspended lung cells, it is possible that recognition occurs through surface structures common to most, if not all, lung cells. Several types of cells are obtained by the dispersion techniques used here (for example, lung tissue yields endothelial, type I and II epithelial cells, fibroblasts and others) (Fig. 2), but contaminating blood cells such as erythrocytes, platelets, glass-adherent phagocytic cells and so on can be removed by density gradient separation, particle phagocytosis and other techniques. The role of blood cell interactions in the arrest of tumour emboli^{14,21} was not examined here, but we are investigating the adhesion of tumour cells to platelets, lymphocytes and macrophages that affect implantation. The ultimate biochemical determination of the surface structures involved in the adhesion of tumour cells to organ cells is feasible using closely related B16 tumour lines with different recognition and metastatic properties.

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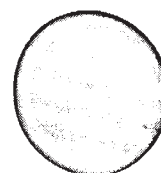
Embryonic use of egg shell calcium in a gastropod

THE function of the calcified outer eggshell found in many gastropod eggs which are deposited on land has been the subject of considerable speculation. Various theories of its relationship to desiccation, mechanical support, protection against predators, physiological buffering and possible calcium source have been discussed¹⁻⁴. This report presents the first quantitative analysis proving that a gastropod embryo uses the egg shell calcium in forming its embryonic shell, the protoconch.

The egg of the land pulmonate *Anguispira alternata* (Stylommatophora: Endodontidae) was examined during its development. This snail deposits eggs which have a heavily calcified, solid CaCO₃ shell made of calcite⁵. Each snail deposits 20-50 eggs, 2.5 mm in diameter, at 20 min intervals during a laying period. Hatching time is approximately 50-60 d at 20 °C; in land pulmonates there is usually a very large amount of variance in the rate of development and consequently in the time of hatching.

The egg shell and egg contents (albumen fluid) were analysed separately for calcium. After 20, 30, and 43 d incubation at 20 °C the eggs were opened and the embryos and egg shells analysed separately for total calcium content (Tables 1 and 2). In the freshly deposited egg, the albumen and single-celled

Fig. 1 Soft X-ray radiograph of a live, newly deposited *Anguispira alternata* egg. Bar represents 2 mm. For the generation of soft X rays, a GE Special Model F-5 machine with a beryllium tube and tungsten target was used. Operating conditions were 25 kV at 5 mA with Du Pont Ortho-Cronar Litho Film.



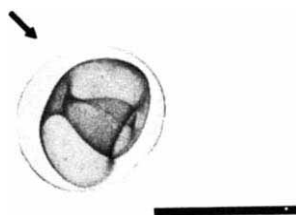


Fig. 2 Egg incubated at 20 °C for 43 d. The egg shell is now thinner and the embryonic shell, the protoconch, has formed at its expense. Arrow indicates where the snail will hatch. Live egg, exposure and printing as for Fig. 1. Bar represents 2 mm.

zygote together contain less than 1% of the total egg calcium; all the rest is in the egg shell. Between 20 and 30 d incubation, the embryo begins to absorb significant amounts of calcium from the egg shell. By 43 d of development, the embryos have absorbed up to 60% of the egg shell calcium. The range of results in Table 2 is a reflection of the staggering of developmental rates in land snails.

Table 1 Mean calcium distribution in the freshly deposited egg

Sample	Sample size	mg Ca per egg
Intact egg (shell + albumen)	5	0.608
Intact egg	5	0.490
Albumen	10	0.003
Albumen	5	0.002

The method of calcium analysis, neutron activation analysis, involves the conversion of a portion of ^{48}Ca , a stable isotope, to ^{49}Ca , which is unstable with a half life of 8.72 min, and measuring the characteristic γ radiation emitted. The source of neutrons was the Ford Reactor in the Michigan Memorial Phoenix Project, at the University of Michigan, the pneumatic tube system providing a flux of about $5 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$. All the samples, in the milligramme range, were placed in polyethylene vials (cleaned with nitric acid) and freeze dried. They were then irradiated for 1 min, allowed to cool for 3 min, the entire vial was then placed near a 30 cm^3 germanium-lithium detector, FWHM 2.3 keV, connected to a 4,096 channel multichannel analyser calibrated to approximately 1 keV per channel with a CoCs source. Each sample was counted for 400 s. The calcium was measured using the 3,083 keV line of ^{49}Ca with reagent grade CaCO_3 powder as the standard. Calculations of the statistical counting errors and variability of controls and standards indicate a possible error of $\pm 10\%$. Whether these minute (2 mm) and very thin (30 μm) egg shells were crushed or not, made no difference in the results at this level of analysis.

Figures 1 and 2 show X-ray radiographs of a freshly deposited egg and an egg incubated for 43 d respectively. Both eggs were placed on the same piece of film, exposed and then printed together. Note that whereas the egg shell is made of calcite, the protoconch is made of another polymorph of CaCO_3 , aragonite. The arrow in Fig. 2 indicates the area in which the embryo has most greatly weakened the egg shell, and at which it will break out. The mechanism of egg shell dissolution and reabsorption is not clear. When the embryo hatches, it consumes any egg shell remnants which are left.

There is evidence that this process of egg shell dissolution occurs in many other families of snails⁶. Calcified eggs have also been reported from land archaeogastropods⁷ and from some of those mesogastropods which deposit their eggs on land⁸. It is suggested that land snails evolved the calcified egg shell early in their ancestry. For these terrestrial animals, calcium is often the limiting factor⁹. It would seem to be of great advantage to have the body shell already calcified by the time of hatching, for protection against small predators.

Embryonic absorption of egg shell calcium has been documented in some insects¹⁰, some reptiles and birds¹¹. A study of the calcium mobilisation in gastropod embryos with these

Table 2 Calcium distribution in eggs incubated at 20 °C ± 1

No. of days incubated	mg Ca in egg shell	mg Ca in embryo	%Total Ca in embryo
20	—	0.003*	—
30	0.601	0.126	17
30	0.510	0.190	27
30	0.646	0.126	16
43	0.285	0.366	56
43	0.326	0.218	40
43	0.601	0.126	17
43	0.338	0.246	42
43	0.523	0.146	22
43	0.211	0.346	62
43	0.646	0.126	16
43	0.253	0.302	54
43	0.510	0.190	27
43	0.522*	0.299*	36*

* Ten specimens combined in a single analysis.

other groups would be most rewarding from a comparative point of view.

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Depressant action of TRH, LH-RH and somatostatin on activity of central neurones

THREE structurally identified hypothalamic-adenohypophyseal regulatory peptides—thyrotropin-releasing hormone (TRH)^{1,2}, luteinising hormone releasing hormone (LH-RH)^{3,4} and growth hormone-release inhibiting hormone (somatostatin or SRIF)⁵, have now been localised both in extra-hypothalamic regions of the central nervous system (CNS)^{6–9} and in certain extraneural regions, such as the pineal gland¹⁰. There is increasing evidence that these peptides may affect behaviour by direct action on the brain. Systemic administration of TRH to rats induces behavioural effects independent of the pituitary–thyroid axis¹¹; LH-RH can induce mating behaviour in hypophysectomised female rats¹², and somatostatin potentiates the behavioural effects of d,l-dopa¹³. These findings together with evidence of high affinity binding sites for TRH in extrahypothalamic tissues¹⁴, identification of LH-RH in synaptosome fractions of brain¹⁵ and localisation of LH-RH^{16,17} and somatostatin^{18,19} in nerve terminals suggest a role for these peptides in neuronal function. Applying TRH, LH-RH and somatostatin directly to central neurones microiontophoretically, we have found that these peptides have a potent depressant action on the activity of neurones at several levels (cerebral and cerebellar cortex, brain stem and hypothalamus) of the neural axis.

Experiments were performed on male Sprague Dawley rats anaesthetised with urethane (1.25 mg kg⁻¹ intraperitoneally in 25% w/v solution) or pentobarbital (50 mg kg⁻¹ intraperitoneally with supplemental intravenous doses). In experiments in which extracellular records were obtained from neurones in the cerebral or cerebellar cortices or the cuneate

Table 1 Summary of depressant responses observed from central neurones during microiontophoresis of hypothalamic releasing (or release inhibiting) peptides

	Site	No. tested	No. responsive	Effective current range* (nA)
TRH	Cuneate nucleus	43	12 (28%)	6–20
	Cerebellar cortex	66	23 (42%)	5–30
	Cerebral cortex	36	17 (47%)	15–25
	Hypothalamus (HVM)	50	30 (60%)	10–18
LH–RH	Cerebellar cortex	17	13 (76%)	5–25
	Hypothalamus (HVM)	16	13 (81%)	10–30
	Cerebellar cortex	9 (L) 9 (C)	5 (55%) 5 (55%)	7.5–20 10–25
Somatostatin	Cerebral cortex	13 (L) 15 (C)	11 (84%) 12 (80%)	10–30 10–30
	Hypothalamus (HVM)	14 (C)	11 (78%)	15–35

All other neurones were unresponsive. Somatostatin was tested in both the linear (L) and cyclised (C) form. HVM, hypothalamic ventromedial nucleus.

* Range of iontophoretic currents which yielded clear depressant effects within 10 s of application. The range of values for either the peptides or the areas does not differ significantly.

nucleus, exposed surfaces were irrigated continuously with mammalian Ringer solution. The hypothalamus was exposed by a retropharyngeal approach, and neurones in the ventromedial nucleus were identified by a characteristic orthodromic response to stimulation of the amygdala or stria terminalis²⁰. Single unit activity was recorded with micropipettes filled with 3.0 M NaCl and the frequency of spike discharges, selected with a voltage gate, was monitored on a polygraph. For microiontophoresis, five or seven-barrelled micropipettes were fixed rigidly to the side of the recording micropipette, and the tips were separated by 10–15 μ m. Solutions of TRH, LH–RH or somatostatin dissolved in distilled water (5 mM) were placed in microiontophoretic channels and ejected as the cation. Adjacent channels always contained sodium L-glutamate (1.0 M, pH 7.0) to excite otherwise silent neurones, and sodium chloride (10 mM) as a current control.

In each area of the CNS studied, only a certain population of neurones responded to microiontophoretic application of individual peptides (Table 1). In responsive neurones, all three peptides depressed discharge frequency (Fig. 1) often in association with an increase in spike size, suggesting membrane hyperpolarisation. For some cells, iontophoretic currents as low as 5–10 nA effected a 50% reduction in spike discharge frequency of both spontaneous and glutamate-evoked activity. The characteristics of the responses in sensitive cells were similar with each peptide: brevity in onset after application of current, rapid recovery after cessation of the ejection current, graded decrease in excitability with increased current, reproducibility with successive appli-

cations and lack of similar responses during application of positive control currents (sodium ions). The spike discharge frequency of insensitive neurones showed no consistent change when peptides were applied even with currents as high as 100–160 nA.

Twelve of fourteen peptide-sensitive neurones (six in the cerebellar cortex, four in the cerebral cortex, four in the hypothalamus) were sensitive to both TRH and LH–RH (Fig. 2b) although the two remaining neurones were responsive to LH–RH only (Fig. 2a). TRH sensitive neurones, more frequent in hypothalamus than elsewhere in the brain, were also found in the cerebellar cortex although this area has a low content of TRH⁹ and lacks high affinity TRH binding sites¹⁴. A wide range of TRH responsiveness for neurones in any one area of the CNS has been reported²¹. As indicated in Table 1, a substantial number of neurones was responsive to LH–RH and somatostatin even in areas where these peptides have either not been detected or are low in concentration. Somatostatin was effective when applied in either the linear or the cyclised (dihydrosomatostatin) form.

Our observation that hypothalamic peptides have potent depressant actions on the excitability of a certain population of neurones in several areas of the CNS extend studies which indicate a depressant action of TRH^{21,22} and LH–RH^{22,23} when applied to hypothalamic neurones; it seems, however, that this response pattern is not solely restricted to hypothalamic neurones involved in pituitary regulation. This more generalised distribution of peptide-sensitive neurones supports the hypothesis that these peptides are involved in

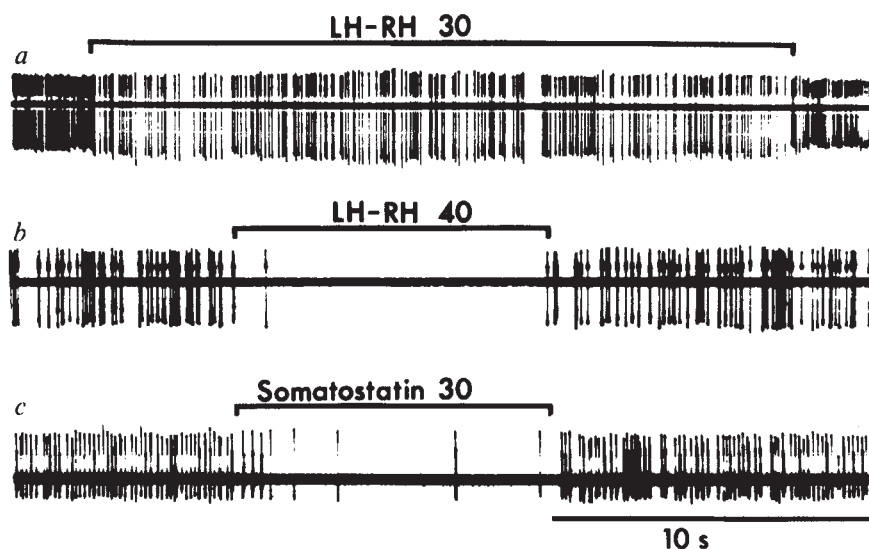


Fig. 1 Representative continuous oscillograph traces to indicate effects of microiontophoresis of LH–RH and somatostatin. Note the increase in spike amplitude (a) and decrease in spike discharge frequency which accompanies applications of LH–RH to two hypothalamic neurones located in the ventromedial nucleus (a, b) and cyclised somatostatin to a neurone in the parietal cortex (c). Numbers above each bar indicate the microiontophoretic current (in nA) used to apply each hormone. Time calibration, 10 s.

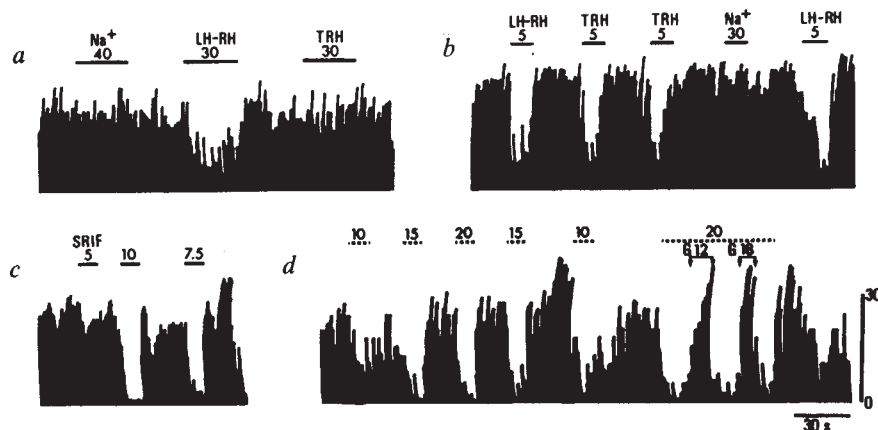


Fig. 2 Polygraph traces of changes in spike discharge frequency of three spontaneously active cerebellar cortical neurones (a-c) in response to microiontophoresis of TRH, LH-RH and somatostatin. Cell a was responsive to LH-RH but not to either TRH or positive current. The firing frequency of cell b was depressed by both TRH and LH-RH. The tracing in (c) indicates a neurone the activity of which was depressed by cyclised somatostatin. d, Linear somatostatin depressed the spontaneous activity of a parietal cortical neurone. This depression remained stable during longer periods of application and could be overcome by brief applications of L-glutamate (arrows). Vertical calibration bar indicates c.p.s.

the modulation of central neuronal activity. Microiontophoretic studies with substance P, another peptide found in the hypothalamus, indicates a predominantly excitatory action when applied to neurones elsewhere in the brain²⁴. Microiontophoretic application of angiotension II was also reported to excite central neurones²⁵.

An understanding of the role of hypothalamic peptides in neuronal function requires data on their ultrastructural localisation and demonstration of their release from synaptic nerve terminals after stimulation. In support of the latter possibility is recent electrophysiological evidence that axons of certain tuberoinfundibular neurones have two (or more) collaterals, one of which terminates on the portal vessels of the median eminence, while the other either terminates locally within the hypothalamus (anterior hypothalamic-preoptic area) or exits from the hypothalamus to other regions of brain²⁶. Thus it is possible that hypothalamic releasing or release-inhibiting peptides are liberated simultaneously both at central synapses as well as at the median eminence terminals of tuberoinfundibular neurones, thereby acting both as neural transmitters and as neurohormones, an earlier suggestion for another hypothalamic peptide, vasopressin²⁷.

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Dopamine depolarising response in a vertebrate neuronal somatic cell hybrid

THERE are various lines of evidence that the catecholamine dopamine (DA) is a neurotransmitter in both invertebrate and vertebrate nervous systems¹⁻⁴. In support of this concept, we report depolarising responses to DA caused by a conductance increase and detected by intracellular recording from a vertebrate somatic cell hybrid of neuronal origin.

Somatic cell hybrids are derived from the fusion of two cell types, producing a dividing cell population that expresses properties contributed by each of the parent cells⁵⁻⁶. Hybridisation of cells of neuronal origin has resulted in clonal lines that express several neuronal characteristics⁷⁻⁹. We used 108CC-15 (also designated NG108-15), which is a somatic cell hybrid of the mouse neuroblastoma N18TG2 and the rat glioma C6-BU-1. Although one parent was of glial origin, the hybrid has numerous properties of nerve cells such as neurone-like morphology, dopamine- β -hydroxylase activity, choline acetyltransferase activity, dense-core and clear vesicles and sensitivity to acetylcholine¹⁰⁻¹². Thus for descriptive purposes the hybrid cell is neuronal and can provide data relevant to the role of DA in an organised nervous system. The advantage of 108CC-15 for investigating receptor mechanisms are: (1) it provides a large homogeneous cell population free of the anatomical complexity of organised tissues; (2) there is no extrinsic synaptic input, facilitating study of single units with DA receptors; (3) the cells are in an isolated, controlled environment that can be easily manipulated, and (4) the cells are large (40-70 μ m) and intracellular recordings can be made with relative ease.

The average membrane potential of 111 cells was -42.5 mV with an average input resistance of 39 M Ω . Detailed analysis of the electrical properties of this cell line has been presented elsewhere¹³.

Figure 1A shows the membrane response to graded amounts of DA. Sixty-four per cent of the 111 cells penetrated gave a depolarising response to DA with an associated decrease in resistance (increase in conductance). The rise time of the response varied with the proximity of

the DA iontophoretic electrode to the membrane. 3-Methoxytyramine-HCl (Sigma) was also applied iontophoretically so as to rule out a response elicited by nonspecific pH effects or a physical stimulus from the current. 3-Methoxytyramine caused no potential or conductance changes in five cells tested but an equal charge of DA gave a response as described above, adding credence to the specificity of the receptor for DA. Repeated applications of DA at short intervals resulted in membrane desensitisation and temporary reduction or loss of the response (Fig. 1B). Similar findings have been reported for the excitatory DA response in *Aplysia* neurones¹⁴. Iontophoretically applied noradrenaline (NA) depolarised the membrane but the response amplitude was less than that produced by an equal iontophoretic charge of DA. In addition, the membrane became desensitised to DA when NA was applied repeatedly. These results indicated that DA and NA had common receptors, but that DA was more efficacious. Other investigators using 108CC-15 have found that NA and DA

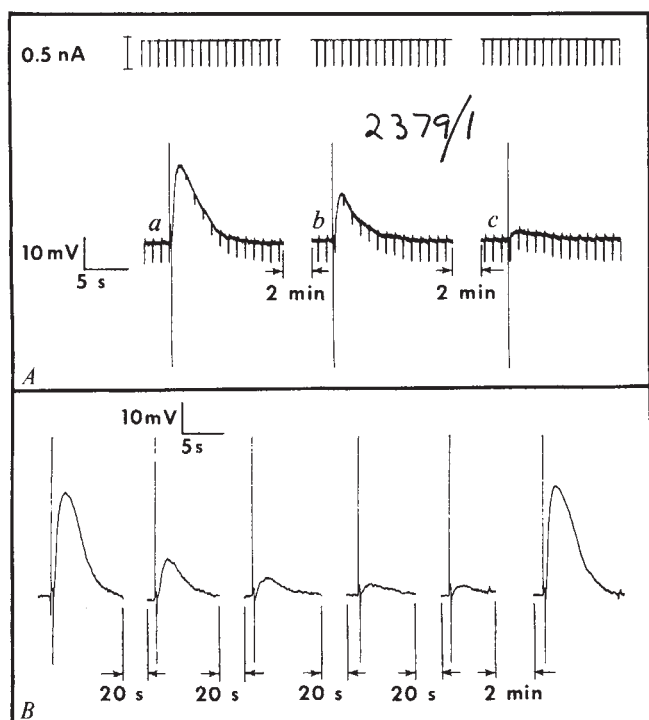


Fig. 1 A, DA depolarising response with a conductance increase. Top trace: current pulses passed across the membrane. Lower trace: dose-response characteristic of iontophoretically applied DA obtained by varying the amount of charge delivered. a, 600 nC; b, 300 nC; c, 100 nC. Current pulses indicate the change in resistance. Cells were grown in Falcon dishes with modified F12 medium¹⁸ supplemented with 5% foetal calf serum, hypoxanthine (30 μ M increased to 100 μ M), aminopterin (0.4 μ M) and additional thymidine (3.0 μ M increased to 16 μ M) at 37 °C and 5% carbon dioxide: 95% air in a water saturated atmosphere. For experiments, 10^4 cells were seeded into 35mm Falcon dishes and grown for at least 7d in medium free of aminopterin but supplemented with 1 mM dibutyl cyclic adenosine monophosphate (Sigma). The drug induces morphological and biochemical differentiation of neuroblastoma cells in culture^{11,19-22}. Standard electrophysiological techniques using a bridge circuit were used to record membrane potential while passing current intracellularly. Glass microelectrodes filled with potassium acetate and potassium chloride and resistances greater than 30 M Ω were used for intracellular recording. For iontophoresis, 1 M dopamine-HCl (Sigma) was dissolved in distilled water and used to fill glass micropipettes (electrode resistance 10–20 M Ω). During recording, culture dishes were maintained at 37 °C and gassed with a stream of water-saturated 10% CO₂: 90% air. B, Desensitisation of the receptor by DA. Iontophoretically applied DA was delivered at short time intervals to demonstrate reduction in response amplitude and full amplitude recovery after a longer time interval. Iontophoretic charge was 500 nC.

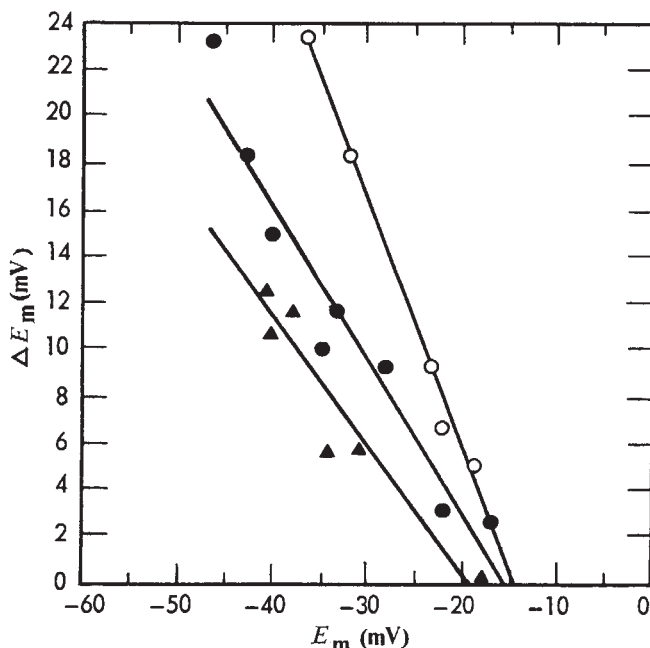


Fig. 2 Reversal potential of the DA response for three cells. Depolarising current was passed across the cell membrane to alter the resting membrane potential. Equal amounts of DA were iontophoretically applied at selected membrane potentials and the response amplitude was determined. Lines were established by linear regression analysis of the data for each cell. The intercept at the abscissa represents the reversal potential.

both depolarise the membrane (J. Traber, G. Reiser, K. Fischer and B. Hamprecht, personal communication).

Figure 2 is a plot of DA response amplitude compared with membrane potential for three individual cells. The intercept at the abscissa was -15.9 mV (± 1.2 ; $n=4$) and represents the DA reversal potential. This value is similar to that found in vertebrate neuromuscular junctions where the acetylcholine response is mediated by an increase in sodium and potassium conductances¹⁵.

Phentolamine, propranolol, chlorpromazine and bulbo-capnine blocked the DA response when present in concentrations greater than 50 μ M, which agreed with their known central actions. Phentolamine and propranolol are adrenergic blockers. Chlorpromazine is a phenothiazine which interferes with DA receptors¹⁶ and bulbo-capnine is a DA antagonist similar in structure to the agonist apomorphine¹⁷.

These studies show that vertebrate cells of neuronal origin can exhibit depolarising responses to DA associated with a conductance increase. Cell preparations of this sort may be of further use in studies of receptor mechanisms in an isolated, controlled environment.

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Nerve terminal ATPase as possible trigger for neurotransmitter release

WHEN a wave of depolarisation spreads down an axon and invades the nerve terminals a number of events rapidly occur and it is difficult to establish the one ultimately responsible for the release of neurotransmitter. There is strong evidence to support the contention that calcium ion influx is involved both in the release of acetylcholine¹ and of noradrenaline², but ion-exchange processes at the level of the vesicle, not necessarily directly involving calcium have been proposed³, and the concept of regulation of release through changes in the activity of an adenosine triphosphatase (ATPase) has also been invoked^{4,5}. This paper is concerned with the last possibility. Following the inward movement of sodium ions, the inward movement of calcium ions to the enzyme on the inner edge of the nerve terminal membrane would be expected to inhibit the activity of sodium, potassium-activated, magnesium-dependent ATPase (Na,K-ATPase)⁶. Sodium depletion, or the addition of ouabain to preparations *in vitro* would also be expected to inhibit Na,K-ATPase activity. These three conditions of enzyme inhibition have all been shown to result in increased acetylcholine release^{4,5}.

We have already suggested elsewhere⁷⁻⁹ that nerve ending particles (synaptosomes) prepared from rat cerebral cortex contain an ouabain-insensitive component of that ATPase activity which is apparently activated by sodium and potassium ions in the presence of magnesium ions, and which is generally assumed to represent Na,K-ATPase. This apparent Na,K-ATPase activity was calculated by subtracting the magnesium-ATPase (Mg-ATPase) activity, determined by measuring the release of inorganic phosphate from Tris-ATP (4 mM) in 50 mM imidazole-HCl buffer pH 7.4 containing 5 mM MgCl₂, from the total ATPase activity determined in a similar mixture which also contained NaCl (150 mM) and KCl (10 mM). The reaction time was 10 min in each case and sodium dodecylsulphate (0.8%) was used to stop the reaction⁹. The ouabain-insensitive component, the existence of which has recently been supported by others^{10,11}, is the result of sodium-activated, magnesium-dependent ATPase (Na-ATPase) activity⁹, determined by measuring the hydrolysis of ATP in the MgCl₂-containing buffer to which NaCl (150 mM) has been added. Note that in synaptosomes, at least, the magnitude of this activity varies with the species of animal, the values for the rat, mouse and guinea pig being 33, 13 and 43% of the apparent Na,K-ATPase activities, respectively.

The implications of the existence of a Na-ATPase together with a true Na,K-ATPase (the ouabain-inhibited enzyme requiring sodium and potassium ions together) in the nerve terminals are considerable if the hypothesis is proposed that inhibition and stimulation of the activities of ATPases are involved in the regulation of neurotransmitter release. We report here data concerning the effects of noradrenaline, phentolamine and acetylcholine on the activities of these enzymes in synaptosomes prepared from rat cerebral cortex as described previously⁹.

Noradrenaline (5×10^{-5} – 1×10^{-3} M) stimulated both the Na- and the Na,K-ATPase activities of the synaptosomes

Table 1 Effects of noradrenaline, phentolamine and acetylcholine on ATPase activities of synaptosomes prepared from rat cerebral cortex

Drug	Concentration (M)	Relative ATPase activities*	
		NaK	Na
None		100	100
Noradrenaline	1×10^{-6}	107.0 ± 3.9(5)	96.1 ± 6.4(5)
	5×10^{-5}	132.5 ± 2.5(2)	135.0 ± 6.9(2)
	1×10^{-4}	141.3 ± 3.0(3)	136.3 ± 16.3(3)
	1×10^{-3}	145.2 ± 5.9(5)	139.3 ± 10.0(5)
Phentolamine	1×10^{-5}	100.0 ± 2.0(2)	97.4 ± 1.7(2)
Phentolamine	1×10^{-5}	99.8 ± 2.6(2)	102.4 ± 2.4(2)
Noradrenaline	5×10^{-5}		
Acetylcholine	1×10^{-5}	86.8 (1)	97.5 (1)
	1×10^{-4}	58.5 ± 2.6(2)	100.9 ± 3.4(2)
	1×10^{-3}	60.1 ± 0.7(3)	97.1 ± 6.7(3)

Numbers of observations in parentheses.

*Results expressed as percentages of control values typically $6.4 \pm 0.5(21)$ and $3.3 \pm 0.3(22)$ μ mol inorganic phosphate per mg protein per h for the Na,K- and the Na-ATPases, respectively. Percentage values under the different conditions have been calculated by comparison with paired controls of the same series.

(Table 1). Other workers^{10,11} have also detected stimulation of Na,K-ATPase by noradrenaline and our results indicate that the Na-ATPase is affected to approximately the same extent as the Na,K-ATPase. Moreover, stimulation of the two enzymes by noradrenaline was decreased by approximately the same extent by the α -adrenoceptor antagonist phentolamine (10^{-5} M, lower concentrations being less effective). In direct contrast to the effect of noradrenaline, acetylcholine (10^{-4} – 10^{-3} M) inhibited the Na,K-ATPase but the activity of the Na-ATPase was not altered in the conditions used.

These results should be considered in the light of evidence suggesting that noradrenaline released from the nerve terminal can cause feedback inhibition of further release^{12,13}. Nerve terminal ATPases may be involved in this mechanism. Thus, although inhibition, of Na,K-ATPase by calcium ions, for instance, may increase neurotransmitter release, stimulation of this enzyme, or of a closely related one, may depress it. The effect of phentolamine in preventing the stimulation of both the Na,K-ATPase and the Na-ATPase by noradrenaline is then compatible with the proposition that feedback inhibition is mediated through α -adrenoceptors^{12,14}.

Although stimulation of both Na- and Na,K-ATPase could be involved in noradrenaline-mediated depression of noradrenaline release, any modulation of this release at synapses by acetylcholine is likely to be through effects on Na,K-ATPase alone (Table 1), inhibition of the enzyme presumably increasing noradrenaline release. Whether or not inhibition of Na,K-ATPase by acetylcholine should be viewed in the light of the suggestion of Langer¹⁵ that presynaptic nicotinic and muscarinic receptor activation can influence noradrenaline release, or in the light of the cholinergic link hypothesis of Burn and Rand¹⁶ is a matter for conjecture; but at least the change in the activity of the enzyme is in the right direction to permit reconciliation with these ideas as well as with the better documented evidence linking increased neurotransmitter release with such factors as calcium ion entry.

Modulation of neurotransmitter release is also believed to be an important function of prostaglandins. Hedqvist¹⁷ showed that the noradrenaline release associated with electrical stimulation of nerves supplying the perfused cat spleen was inhibited by prostaglandins of the E series and he later found that the inhibitory effect of prostaglandin E₂ (PGE₂) was antagonised by calcium ions¹⁸. We have

observed⁹ that PGE₁ stimulates synaptosomal Na,K-ATPase activity. Here again it could be assumed that stimulation of the Na,K-ATPase inhibits neurotransmitter release, and that the inhibitory effect of calcium ions on the activity of the enzyme antagonises this process.

Evidence for a more direct association between the activities of ATPases in the nerve terminals and the regulation of neurotransmitter release should be obtained before the hypothesis is strongly supported. The mechanism of regulation by the enzymes requires consideration although one could propose that a change in the local environment of a membrane-bound enzyme results in both a change in its activity and its conformation such that an increase in exocytosis occurs. It could be argued, however, that the rates of activity of the enzymes are not sufficiently high to regulate neurotransmitter release to a fine degree by such a mechanism. Again, the observations mentioned here in connection with the hypothesis are drawn from work involving both peripheral nerves and the central nervous system, and the information concerning the effects of drugs on the activities of the ATPases has come from work with cerebral cortex synaptosomes which contain at the least both cholinergic and adrenergic types. We feel, nevertheless, that the hypothesis merits further attention.

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Oxygen control of cytochrome *c* oxidase synthesis in isolated mitochondria from *Saccharomyces cerevisiae*

DURING anaerobic growth *Saccharomyces cerevisiae* cells produce respiratory-deficient mitochondria-like structures—promitochondria—which contain a normal complement of mitochondrial DNA (mtDNA) and many mitochondrial enzymes but which lack cytochrome components of the inner mitochondrial membrane^{1–4}. On exposure to oxygen these respiratory-deficient structures develop a full complement of cytochromes and differentiate into respiring mitochondria⁵. Although the precise mechanism by which oxygen mediates mitochondrial biogenesis is still incompletely understood recent studies with whole yeast cells have shown that one effect of oxygen is at the level of either synthesis or assembly of some mitochondrial translation products^{6–8}. Unfortunately, these *in vivo* studies could not localise the primary effect of oxygen to the mito-

Table 1 Effect of anaerobiosis on the incorporation of ¹⁴C-leucine into isolated mitochondria

Additions or omissions	Incorporation (c.p.m. per mg protein)	
	Aerobic	Anaerobic
None	3,500	2,680
+ Chloramphenicol	314	258
+ Cycloheximide	3,550	—
— ATP, PEP, pK	—	1,000

Protein synthesis in isolated mitochondria was measured as described previously¹⁰. Anaerobic incubations were carried out in long test tubes with the reaction mixture covered with liquid paraffin. Five minutes after the oxygen was consumed by the respiring mitochondria (usually in 1–1.5 min as checked with a Clark oxygen electrode in a parallel incubation) the reaction was started by the addition of 0.2 μ Ci ¹⁴C-leucine (60 mCi mmol⁻¹) per ml by means of a syringe fitted with a long needle.

chondrion nor could they demonstrate that oxygen acts directly on mitochondrial protein synthesis rather than indirectly by inhibiting other biochemical reactions, for example in the biosynthesis of haem⁹, required for the development of functional cytochromes. As a first step towards localising the effect of oxygen and ascertaining if it is indeed at the level of the mitochondrial protein synthesis we have studied the effect of O₂ on the *in vitro* synthesis of three mitochondrial translation products, subunits I, II and III of cytochrome *c* oxidase, by isolated mitochondria. We have found that mitochondria incubated in the presence of oxygen synthesise subunits I, II and III and assemble them with the four cytoplasmically-made subunits⁸ into a holoenzyme¹⁰, although mitochondria incubated in the absence of oxygen do not synthesise subunits I and II. These results demonstrate that oxygen acts directly on the mitochondrion and strongly suggest that at least one step in mitochondrial transcription-translation requires oxygen.

Table 1 summarises the effect of anaerobiosis on the incorporation of radioactive leucine into acid-insoluble material by isolated mitochondria. Both aerobic and anaerobic incorporation show the expected response to chloramphenicol and cycloheximide, indicating that essentially all of the protein synthesis is taking place on mitochondrial ribosomes. Incorporation of radioactive leucine into protein in anaerobic conditions is dependent on the continuous supply of ATP by the ATP-regenerating system, phosphoenolpyruvate–pyruvate kinase, and is 50–70% (4 experiments) of that incorporated in aerobic conditions.

The effect of oxygen on the *in vitro* synthesis of subunits I, II and III of cytochrome *c* oxidase was assessed by determining the amount of radioactivity precipitated from mitochondrial extracts by an antiserum to cytochrome *c* oxidase and analysing the immuno-precipitates by sodium dodecyl sulphate (SDS) gel electrophoresis. The immunotitration

Table 2 Effects of anaerobiosis on the labelling of cytochrome *c* oxidase

Labelling condition	Experiment	Specific activity of labelled mitochondria (c.p.m. per mg protein)	Total mitochondrial label immuno-precipitated (%)
Aerobic	1	4.85×10^4	8.7
	2	3.33×10^5	10.4
Anaerobic	1	2.85×10^4	4.2
	2	1.59×10^5	6.0

Isolated mitochondria were pulse-labelled with 0.1 mCi ml⁻¹ of L-4,5-³H-leucine (3Ci mmol⁻¹) for 20 min either aerobically or anaerobically as described in Table 1. The labelled mitochondria were solubilised with cholate-KCl and subjected to immunotitration using an antiserum to holo-cytochrome *c* oxidase as described previously¹⁰. Values for the percentage of total mitochondrial label immunoprecipitated represent plateau values taken from immunotitration curves and have been corrected for nonspecific precipitation obtained with control serum.

results shown in Table 2 indicate that labelling of cytochrome *c* oxidase in anaerobic conditions is reduced by about 50% relative to the aerobic control. Electrophoretic analysis (Fig. 1*a* and *b*) of the immunoprecipitates from aerobically and anaerobically-labelled mitochondria reveals that subunit III of cytochrome *c* oxidase is labelled both in the presence and absence of oxygen, but that subunits I and II are labelled only in the presence of oxygen. Since subunits I and II account for 40–50% of the radioactivity in immunoprecipitates from aerobically-labelled mitochondria it seems that the decreased level of anaerobic incorporation is caused solely by the lack of synthesis of these two subunits. This finding that subunits I and II are

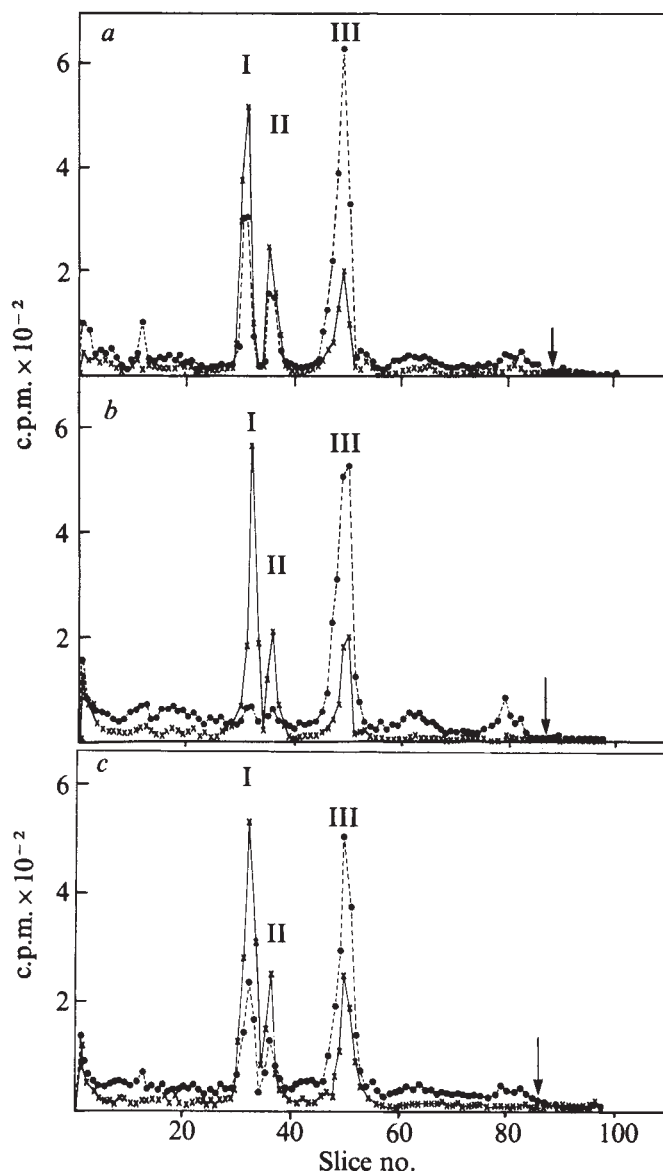


Fig. 1 SDS polyacrylamide gel analysis of cytochrome *c* oxidase immunoprecipitates from extracts of mitochondria labelled: *a*, aerobically; *b*, anaerobically; and *c*, aerobically after a 20 min anaerobic preincubation. Isolated mitochondria which were labelled *in vitro* with ^3H -leucine either aerobically or anaerobically were solubilised in cholate-KCl, mixed with mitochondrial extracts from cells labelled *in vivo* with ^{14}C -leucine in the presence of cycloheximide and subjected to immunoprecipitation¹⁰ using an antiserum to holocytochrome *c* oxidase. The immunoprecipitates were solubilised in SDS and electrophoresed on 12% acrylamide–0.375% bis-acrylamide gels as described previously¹³. The arrow marks the position of the dye front. $\times$, ^{14}C -leucine counts immunoprecipitated from *in vivo* labelled mitochondria (cells + cycloheximide); $\bullet$, ^3H -leucine counts immunoprecipitated from *in vitro* labelled mitochondria.

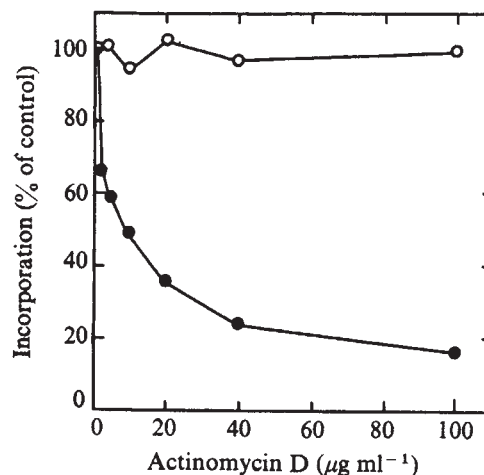


Fig. 2 Effect of actinomycin D on RNA and protein synthesis in isolated mitochondria. Mitochondria (2 mg) were incubated as described previously¹⁰ and pulse-labelled for 20 min with $50 \mu\text{M}$ ^3H -UTP (10^8 c.p.m. nmol^{-1}) and $5 \mu\text{M}$ ^{14}C -leucine (0.04×10^8 c.m.p. nmol^{-1}). Acid-insoluble counts were measured. The 100% value corresponded to 5 pmol ^{14}C -leucine ($\circ$) and 0.5 pmol ^3H -UTP ($\bullet$) incorporated per mg protein per min.

synthesised *in vitro* only in the presence of oxygen fully parallels the *in vivo* studies of Mason and Schatz⁸ and, in addition, demonstrates that oxygen can act directly on the mitochondrion.

To exclude the general deleterious effect of the absence of oxygen on mitochondrial protein synthesis, we have tested the reversibility of the oxygen effect. To reproduce *in vitro* the "aerobic adaptation" phenomenon already well documented from *in vivo* studies we incubated isolated mitochondria anaerobically for 20 min and then aerated them in the presence of ^3H -leucine. A comparison of Fig. 1(*c*) with Fig. 1(*a*) reveals that subunits I and II are labelled to near normal levels after the anaerobic preincubation. Thus, it seems that the response of isolated mitochondria to the absence of oxygen is exactly like that of whole cells.

At the moment there seem to be three possibilities to explain the finding that subunit III of cytochrome *c* oxidase is unresponsive to oxygen although subunits I and II are positively controlled by it. First, oxygen may act at the level of mitochondrial transcription either by stimulating an RNA polymerase¹¹ or by inactivating a repressor¹². This explanation assumes that the genetic information for subunits I and II of cytochrome *c* oxidase is present on mtDNA, an assumption for which there is no direct evidence. Second, oxygen may act at the level of mitochondrial translation, and third, oxygen may be required for the integration of polypeptide subunits (for example subunits I and II of cytochrome *c* oxidase) into enzyme complexes. For this explanation to be correct we would have to assume that subunits which are not integrated are rapidly degraded as they cannot be detected by immunoprecipitation. Since we have not observed a substantial increase in low molecular weight polypeptides in immunoprecipitates from anaerobically-labelled mitochondria we consider this explanation unlikely. While we cannot rule out the first possibility, some evidence against it is obtained from Fig. 2. Actinomycin D in a rather high concentration seems to be an effective inhibitor of mitochondrial transcription (inhibiting UTP incorporation up to 80%), whereas at the same concentration no effect is seen on the incorporation of leucine. This result indicates that mitochondrial translation is not dependent on at least the bulk of mitochondrial transcription. It seems unlikely, therefore, that inhibition of mitochondrial transcription would lead to the observed inhibition of incorporation of

label into subunits I and II of cytochrome *c* oxidase. Thus, at the moment, it seems most likely that oxygen is regulating the synthesis of subunits I and II of cytochrome *c* oxidase directly at the level of mitochondrial translation.

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Molecular basis for the α_1 -protease inhibitor deficiency

THE α_1 -protease inhibitor (formerly α_1 -antitrypsin) is inherited by a series of autosomal codominant alleles that determine its concentration and electrophoretic mobility. Serum from individuals with severe α_1 -protease inhibitor deficiency usually contains an electrophoretically slow-moving α_1 -protease inhibitor (type ZZ) in an amount 10-40% of that found in the normal population (type MM). This deficient state is associated with either an early-onset form of familial emphysema or familial infantile cirrhosis¹.

So far, however, the molecular basis for this deficiency has not been established although Bell and Carrell² and Cox³ suggested that the Z protein lacked some of the sialic acid residues found in the normal protein. They reasoned that a glycoprotein with fewer terminal sialic acid residues could be expected to accumulate intracellularly by binding to the plasma membrane of liver cells, thus effectively decreasing the concentration of the circulating protease-inhibitor. This hypothesis is consistent with a report⁴ of patients with severe α_1 -protease inhibitor deficiency (type ZZ) whose serum was markedly deficient in sialic acid transferase activity. We have now found that the Z protein not only contains less sialic acid but also contains smaller amounts of the other carbohydrates than the M protein. Based on the established structures of the two oligosaccharide units present in the M protein, it seems that there are four oligosaccharide units in the M protein and three in the Z protein. We have also observed that the amino acid composition of the Z protein has a higher amount of glycine and arginine than that of the M protein. It is possible that this difference in amino acid sequence affects the attachment of the carbohydrate side chains, leading to a difference in the rate of secretion of the M and Z proteins which is ultimately

responsible for the low concentration of the circulating Z protein in individuals with severe α_1 -protease inhibitor deficiency.

The α_1 -protease inhibitor was isolated from individuals of genetic type MM and ZZ and purified as before⁵ and the genetic typing⁶ was determined by two-dimensional electrophoresis. Since no change in the electrophoretic pattern of either protease inhibitor was observed before or after purification, the procedures for isolation did not alter the protein molecule. The isolated products were free of albumin and orosomucoid as shown by immunodiffusion on agar plates, and were homogeneous when subjected to disc electrophoresis on polyacrylamide gel⁷ at pH 8.9 or 7.0. Results to be published in full elsewhere show that the M protein has a molecular weight of 54,000 and consists of a single polypeptide chain with four carbohydrate side chains of two structural types. The first type consists of four residues of N-acetylglucosamine, three of mannose, three of galactose and three of sialic acid. The second type consists of three residues of N-acetylglucosamine, three of mannose, two of galactose and two of sialic acid. These two types of oligosaccharide unit are present in equal amounts in the M protein.

Table 1 Comparison of the composition of amino acids and carbohydrates of the M and the Z α_1 -protease inhibitor

	mol per 54,000 of	protein
	M	Z
Aspartic acid	43	42
Threonine	33	29
Serine	27	27
Glutamic acid	54	51
Proline	19	19
Glycine	22	30
Alanine	27	27
Valine	24	24
Half-cystine	ND	ND
Methionine	9	9
Isoleucine	18	16
Leucine	44	43
Tyrosine	6	6
Phenylalanine	28	26
Lysine	37	33
Histidine	12	13
Arginine	8	12
Tryptophan	ND	ND
N-acetyl-D-glucosamine	13	11
D-mannose	12	8
D-galactose	9	6
Sialic acid	9	5

Samples of α_1 -protease inhibitor were hydrolysed in the following conditions: for amino acids, 6 N HCl at 110 °C *in vacuo* for 48 h; for N-acetyl-D-glucosamine, 4 N HCl at 100 °C *in vacuo* for 6 h; for neutral sugars, D-galactose and D-mannose, 1 N H₂SO₄ in sealed tubes for 8 h at 100 °C and for sialic acid, 0.18 N H₂SO₄ at 80 °C for 1 h. Amino acids and D-glucosamine were analysed on a Technicon automated amino acid analyser¹⁰, whereas the neutral sugars were determined on an automated sugar analyser according to Lee *et al.*¹¹. Sialic acid was determined as described by Warren¹². Norleucine was used as an internal standard for analysis of amino acids and D-glucosamine, and D-xylose was used for D-galactose and D-mannose. ND, not determined.

Table 1 shows that the mannose content in the Z protein is approximately three-quarters of that of the M protein and suggests that the Z protein contains three instead of four carbohydrate side chains. Indeed, the Z protein contains roughly four sialic acid residues fewer than does the corresponding M protein. This result is consistent with the speculation of Bell and Carrell² and Cox³. The difference in sialic acid residues, however, does not necessarily explain the low concentration of the α_1 -protease inhibitor in circulation since it is the exposure of the non-reducing terminal galactosyl residues that provides a means by which the liver recognises and removes asialoproteins^{8,9}. Thus, even though the M and Z proteins differ in the number of galactosyl and sialic acid residues, as we have shown, the number of

exposed galactosyl residues between the two proteins is essentially the same. If, however, the efficiency in the secretion of liver protein depends on the number of carbohydrate side chains, it is conceivable that the extra carbohydrate chain present in the M protein facilitates its secretion and thus maintains its concentration at the normal level.

Table 1 also compares amino acid composition between the M and Z protein. Because of the relatively large molecular weight of the protein, the data are more difficult to interpret. There was minimal difference ($\pm 10\%$) between the two homologous proteins, except that the Z protein had a significantly larger amount of arginyl and glycyl residues. In this case, the M protein would have been expected to be more negatively charged than the Z protein. This is verified by comparing the electrophoretic mobilities of these two proteins and their corresponding asialoproteins. The mobility of the Z protein toward the anode was considerably slower than that of the M protein. After the removal of the sialic acid residues by digestion of the proteins with neuramidase (5% by weight) at 38 °C for 16 h, the electrophoretic mobilities of both proteins were retarded, but the asialo Z protein maintained a slightly slower mobility than the asialo M protein, consistent with the amino acid composition data shown in Table 1.

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Non-random occurrence of 7-14 translocations in human lymphocyte cultures

SOMATIC cells dividing *in vitro* occasionally show chromosome recombination between non-homologues, most often in situations associated with an increased frequency of chromosome breakage and reunion^{1,2}, their occurrence being recognised by the observation of exchange figures. With the advent of new techniques for chromosome identification³, it is now possible to identify the chromosomes involved in these rearrangements and to recognise some translocations which might otherwise be missed.

We have observed about 5,000 trypsin-banded⁴ metaphases, from 250 individuals, among which a total of 9 cells seem to have a *de novo* balanced translocation. (The 'true' denominator needed to determine the percentage of abnormal cells of this type is necessarily imprecise, since in the course of scanning a preparation, attention may be drawn to a somewhat imperfectly banded metaphase with a morphologically abnormal chromosome and/or a unique banding pattern.) These translocations seem to be non-random, since three of the nine abnormal cells involved a translocation between chromosomes 7 and 14 (Fig. 1). These three cells were noted in three different in-

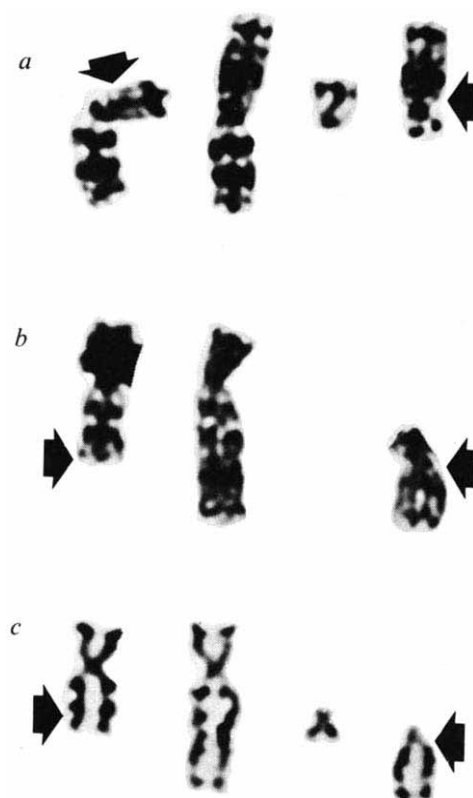


Fig. 1 Partial karyotypes of the three individuals found to have 7-14 translocations. The arrows indicate the breakpoints believed most likely for the number 7 and 14 chromosomes (see text). Note that there was no residual small acrocentric present in cell b. (Cell c was noted after routine Giemsa staining; it was subsequently destained and trypsin banding carried out.) The chromosomes have been arranged to match homologous segments whenever possible.

dividuals, studied for disparate reasons: one had Rubinstein-Taybi syndrome, one had multisystem nonspecific congenital anomalies and one was the healthy father of an infant with trisomy 22.

All three 7-14 translocations arose in short term (72-h) lymphocyte culture and the breakpoints of the number 14 chromosomes all seemed to involve the same region, q1(1-2). The breakpoints of the number 7 chromosomes were more variable (Fig. 1); cell a being 7p13; b 7q3(3-6); c 7q3(4-6). In two of the other six translocation lymphocytes noted in this study, a 7 or 14 chromosome was also involved; these were 46,XY, t(2;14)(p11;q32) and 46,XX, t(7;10)(q11-22;q2(4-6)). None of the preparations containing a *de novo* translocation showed any suggestion of an increased frequency of chromosome breakage.

It seems clear from these results that translocations between chromosomes 7 and 14 arise much more frequently during *in vitro* culture than would be expected on a random basis. This finding is corroborated by results in the two following papers^{5,6} in this issue. The culture time allows for the possibility that the breakage and reunion may be an *in vitro* phenomenon only, and although the circumstances suggest that it is not likely to be a result of mycoplasma infection, a viral aetiology cannot be excluded.

The breakpoints involved in somatic *de novo* 7-14 translocations seem to be relatively specific. Combining these data with those of Beatty-DeSana *et al.*⁵ and Hecht *et al.*⁶, we note that the 14q1(1-2) region is involved in all twelve somatic 7-14 translocation cells observed, together with the 7q3(3-6) region in six of these and the 7p13 region in the remainder. None of these breakpoints occurred in the 2-14 and 7-10

single cell translocation noted above. Further, although chromosome 14 is commonly involved in human meiotic translocations⁷⁻⁹, meiotic 7-14 translocations seem to be uncommon and those reported previously^{10,11} involve break-points other than those above.

Our observations could be a result of an increased tendency to breakage in certain regions of chromosomes 14 and 7, together with the relative proximity of these chromosomes during somatic replication. The latter may be associated with the residua of ancient homology between heterologous chromosomes¹² and we think that there is some resemblance between the 14q and 7p banding patterns. This theory would suggest an increased number of 7-14 exchanges following lymphocyte irradiation, however, but this does not occur¹³⁻¹⁵.

Similar 7-14 translocations occurring in proliferative clones⁶ have been noted in ataxia-telangiectasia^{1,6}, a condition often associated with lymphoid neoplasia. Because of the rarity of meiotic 7-14 translocations and the known association of another relatively specific somatic translocation with chronic myeloid leukaemia¹⁶, we wonder whether these observed 7-14 somatic translocations may also have an increased malignant propensity.

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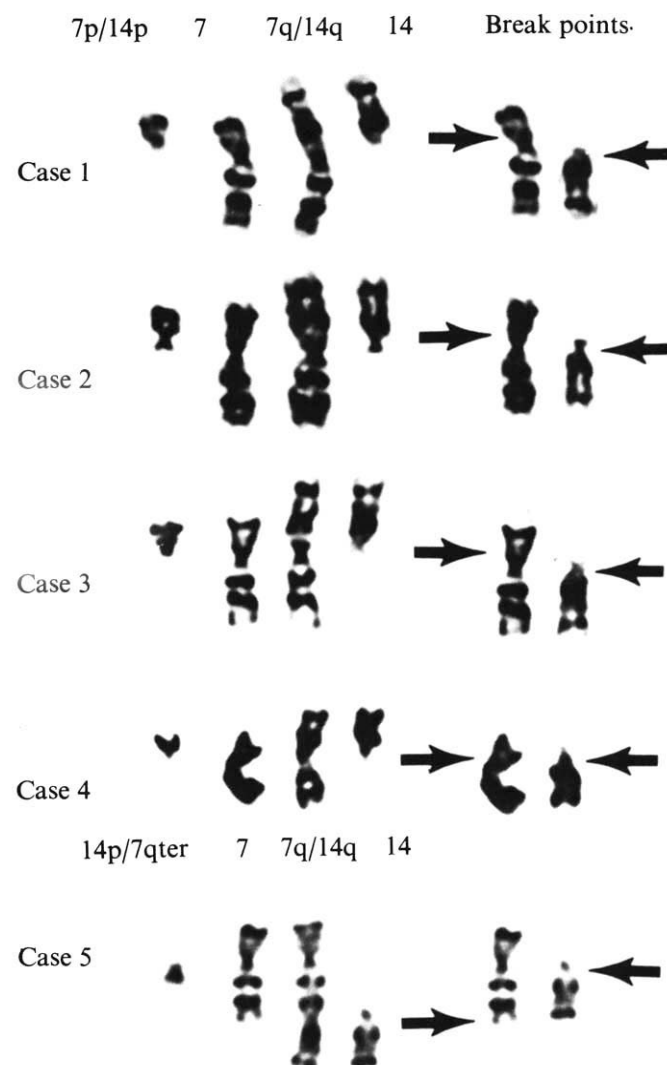
CYTOGENETIC studies of human lymphocytes cultured from patients and their families using known banding methods¹⁻² provide an opportunity to observe spontaneous chromosomal aberrations, to identify the chromosomes involved in a rearrangement and aid in interpreting the cytogenetic events leading to their formation. In our investigations of many high risk families over a 4-yr period, all breaks, fragments and rearrangements noted in the lymphocyte cultures were recorded, along with the total number of cells counted because of previous experience in the study of X-ray induced chromosomal aberrations^{4,6}. The observations reported here are from 72-h cultures, which may complicate their interpretation^{6,7} since they are subject to the selective advantages or disadvantages of a particular chromosomal rearrangement and other factors. The many variables inherent in the analysis preclude rigorous statistical treatment of these data, but a general description of the nature of occurrence may be helpful.

Studies of 25 lymphocyte metaphases each are from 852 consecutively karyotyped mentally retarded people and their families, together with others serving as controls in various

studies. Five unrelated people aged less than 40 with normal karyotypes (three with nonspecific mental retardation and two normal, first-degree relatives of people with chromosome anomalies) were found to have a single cell containing a reciprocal translocation between chromosomes 7 and 14. The apparent break point was at 14q1(1-2) in all five, and at 7p13 in four and 7q3(4-6) in the fifth (Fig. 1)⁸. Amplification of the pale band at the region where the two breaks are thought to have joined in the large translocation chromosome indicates that the break is at the edge of the pale band in each chromosome, so that region 7p13 (or 7q3(4-6) in one translocation) is now adjacent to 14q11. We found a total of 42 two-break rearrangements (1 in 500 cells) including dicentrics and rings—usually with accompanying fragments—translocations and exceedingly rare chromatid exchanges. With the exception of the 7-14 translocation, no other consistent aberration was found. Three others, however, involved D-group chromosomes, two dicentrics with fragments, and a 13-14 translocation, all three of which had breaks seemingly in the 14q11 and telomere regions. The consistent translocations have been found once every 8-10 months during the past 4 yr. No infection of cultures or contaminant of the culture media is suspected.

A sample of 25 cells from a single human represents only 2×10^{-9} of the total lymphocyte population⁹; thus the non-random occurrence of a 7-14 translocation in a single cell of five individuals, not known to have any malignancy or any

Fig. 1 Sporadic reciprocal translocations involving chromosomes 7 and 14 in human lymphocytes.



suggestion of a predisposition to increased chromosome breakage, indicates that this may be a phenomenon more widespread in routine lymphocyte cultures than is now known. Studies from other cytogenetics laboratories reported in this issue^{10,11} provide similar findings which confirm these results. The similarity of these aberrant chromosomes indicates a specificity of breakage points which suggests that their presence is significant and that one or more of the following factors may be involved: A specific aetiological agent¹²; some selective advantage¹³ conferred by the translocation; a genetically-based alteration of the repair mechanism¹⁴; or the spatial arrangement of the chromosomes in the cell. The true significance of these translocations awaits further study.

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We report here the occurrence of somatic chromosome translocations in lymphocytes from individuals studied for clinical cytogenetic diagnosis. We note that these translocations are non-random in composition and resemble those in ataxia-telangiectasia (A-T) lymphocyte clones¹. Our data have been collated with information from two other centres (Halifax and Atlanta) reported in this issue^{2,3}, to provide a preliminary estimate of the frequency of chromosome translocations in cultured lymphocytes.

Our sample consisted of 6,000 lymphocyte metaphases; 20 from each of 300 consecutive patients or relatives referred for genetic counselling. None was known to have a chromosome instability syndrome, malignancy, or unusual X-ray or drug exposure. Lymphocytes were cultured with phytohaemagglutinin (PHA) for 68-72 h and harvested with colchicine. Metaphases were Giemsa-banded, selected under low power microscopy and photographed. Karyotypes were prepared from two or more cells per person⁴.

Four of the 6,000 cells were found to contain a chromosome rearrangement and each of the four was from a different person. Each rearrangement was a translocation between chromosome 7 and 14 (Fig. 1). Three involved the q33-35 region and one the p13 band of chromosome 7. The breakpoints in chromosome 14 were consistently in band q12.

The chromosome rearrangements in this study resemble those observed in A-T lymphocytes. Lymphocyte clones from 22 A-T patients have been detected, each with a translocation. These A-T translocations involve a D chromosome, identified as No. 14, with the breakpoints in band 14q12. The other chromosome in the A-T translocation is often a No. 7 with the breakpoints (ref. 1 and B.K.M., F.H., D. G. Harnden and R. Teplitz, ref. 8) in p13 or q33-35, as in our study. These data suggest that in lymphocyte somatic chromosome rearrangements there is non-random involvement of chromosomes 7 and 14.

Table 1 Frequency of translocations in cultured human lymphocytes

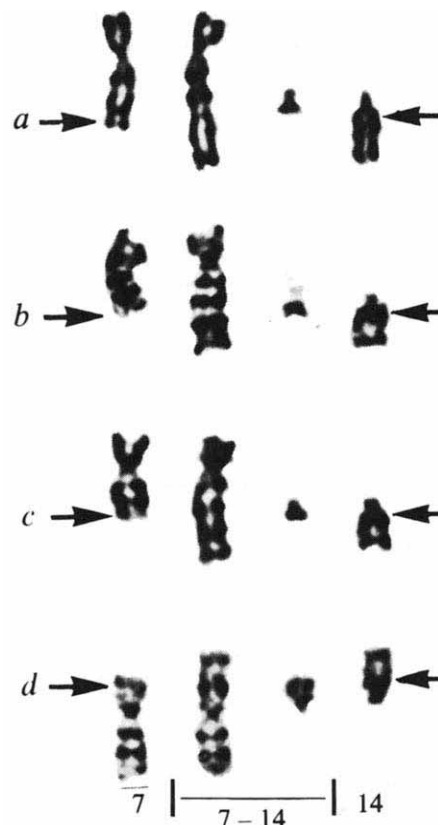
Locale	Individuals	Lymphocytes	All translocations	7-14 translocations
			No. (freq.)	No. (freq.)
Halifax	250	5,000	9 (1/556)	3 (1/1667)
Portland/Denver	300	6,000	4 (1/1500)	4 (1/1500)
Atlanta	852	21,300	42 (1/507)	5 (1/4260)
Total	1,402	32,300	55 (1/587)	12 (1/2692)

From our study and those reported in this issue (refs. 2,3 and Table 1) the frequency of *de novo* chromosome translocations in cultured lymphocytes is calculated to be roughly 1.7×10^{-3} . The frequency of 7-14 translocations is roughly 4×10^{-4} . These are crude estimates, since the sample size is relatively small and subtle rearrangements may have been missed.

By comparison, it is interesting that the mutation rate for structural autosomal rearrangements in germ cells resulting in live births has been estimated to be in the same range, about 4×10^{-4} (ref. 5). It is also interesting that meiotic translocations in humans are generally non-random in composition. For example, D/D translocations are largely of the 13-14 type⁷ and D translocations in patients with Down's syndrome tend preferentially to be of the 14-21 type^{6,7}.

The basis for the non-randomness of somatic chromosome rearrangements is open to conjecture. It must reflect a preferential pattern of rearrangement and/or selection for lymphocytes with 7-14 translocations. The repositioning of genetic material ('position effect') presumably may alter the expression of genes and thus change cell behaviour and function in some as yet unexplained manner.

Fig. 1 Translocations in lymphocytes cultured from four individuals (a-d). The arrow on the left indicates the point at which the distal part of chromosome 14 is translocated to the long arm (a-c) or to the short arm (d) of chromosome 7. The arrow on the right indicates the breakpoint in chromosome 14.



The biological meaning of these translocations in lymphocytes is not yet clear. They may occasionally we believe (B.K.M., F.H., D. G. Harnden and R. Teplitz, ref. 8), represent an early tentative step towards the eventual development of lymphoid malignancy.

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Factor suppressing α -foetoprotein production in newborn mice

α -FOETOPROTEIN (AFP) is one of the main proteins of embryonic serum. Its synthesis begins in the yolk sac, continues in embryonic liver and terminates, in mice and rats, at the beginning of the postnatal period^{1,2}. Presumably, a powerful suppression factor acts on AFP production by embryonic liver cells after the embryonic period has finished. The present study was undertaken to determine this factor(s).

Families of newborn BALB/c mice were divided into two groups. One group was injected daily with serum or extracts of various organs of syngeneic adult mice and embryos, and the other was untreated. After 10 d, the AFP titre was determined in the serum of each group of newborn mice. Extracts of small intestine were used because, during embryogenesis, the liver is formed out of the small intestine and various endocrine connections may persist between these two organs.

Extracts (see Table 1) were injected into the skin of the back of newborn mice, the volume of the material injected increasing as the animals grew older. From day 1 to the day 4 of life, mice were inoculated with 0.1 ml, on days 5 and 6 with 0.2 ml, on day 7 with 0.3 ml, on days 8 and 9 with 0.5 ml. Newborn mice were injected, according to the same schedule, with sterile serum from adult mice. Each mouse killed on the day 10 had received 2.1 ml 20% extract. The AFP titre of controls receiving only Hanks' solution was not affected (Table 1).

Microprecipitation in agar was performed as described previously³. Mouse serum was diluted from 1:2 to 1:1,024. This test system (1:2) was used with the above dilutions of the sera from newborn mice and the results determined on the following day (Fig. 1a). Test system: (1) antigen-serum of newborn mice; (2) antiserum—the monospecific serum of rabbits immunised with purified α -foetoprotein.

It is evident from Table 1 that in the serum, in the small and large intestine, liver, lung, brain, thymus, spleen and kidneys of adult mice there is a factor(s) suppressing AFP production in neonatal mice. Figure 1b and c show the AFP-suppressing effect of the extracts of small intestine. Extracts of 21-d-old mouse intestine produced a weaker effect than those of the adult.

The factor is hardly detectable in small intestine of mice embryos, in the whole embryo or in striated muscles of adult mice. Heating of the small intestine extract at 60 °C for 45 min inactivated the suppression mechanism (data not shown). This high thermostability probably indicates the protein nature of the factor.

The function of embryonic antigens in tumour cells and the factors controlling their appearance remain obscure. Growth of tumours and transplants of foetal digestive tract is inhibited in newborn mice^{4,5}, and it may therefore be anticipated that suppression factor(s) controlling the synthesis of embryonic antigen are produced in the embryonic body, but are absent in

Table 1 AFP titre in the pooled sera of newborn mice inoculated with extracts of various tissues and killed on the day 10

Family number	Experimental Injections with 20% extract of:	Titre*	Control (with- out injection) —titre*
1	Adult small intestine	64	512
2	Adult small intestine	64	512
3	Adult small intestine	32	512
4	Adult small intestine	32	512
5	Adult large intestine	64	256
6	Adult large intestine	64	256
7	Adult large intestine	32	512
8	Adult large intestine	32	512
9	Adult kidneys	32	256
10	Adult kidneys	64	256
11	Adult liver	64	256
12	Adult liver	64	256
13	Adult lung	16	256
14	Adult lung	32	512
15	Adult spleen	64	256
16	Adult spleen	64	512
17	Adult thymus	64	512
18	Adult thymus	128	1024
19	Hanks' solution	256	256
20	Hanks' solution	256	256
21	Adult muscle	128	128†
22	Adult muscle	256	256†
23	Adult brain	64	256
24	Adult brain	64	256
25	Small intestine from 21-d-old mice	64	128
26	Small intestine from 21-d-old mice	64	256
27	Small intestine from 21-d-old mice	64	256
28	Small intestine from 21-d-old mice	64	256
29	Small intestine from embryos	256	256†
30	Small intestine from embryos	128	256
31	Embryos without gastrointestinal tract	512	512†
32	Embryos without gastrointestinal tract	512	512†
33	Adult serum	64	512
34	Adult serum	64	512
35	Hanks' solution	512	512
36	Hanks' solution	512	512

*Reciprocal to last dilution of serum of newborn mice in which AFP is determined in the microprecipitation test.

†Serum AFP titre of the experiment and control was the same, although the intensity of the precipitation line in the control was a little more distinct than in the experimental half.

The upper part (8 cm) of the small intestine was excised from adult BALB/c mice and its contents evacuated. It was then sectioned, washed repeatedly with Hanks' solution containing antibiotics (1000 U penicillin and streptomycin ml⁻¹), incubated in this solution for 30 min at 37 °C, and then washed repeatedly with Hanks' solution without antibiotics. The tissue was carefully ground in a mortar with glass powder; 20% extracts (20 g tissue 100 ml⁻¹ Hanks' solution) were prepared with 200 U ml⁻¹ penicillin and streptomycin, and the homogenate centrifuged at 500 r.p.m. for 5 min. The supernatant was poured into plastic ampules and stored in liquid nitrogen (-196 °C) for 18 h, and subsequently at -22 °C. When required, ampules containing extracts were thawed and used for injections. Sterile extracts from liver, kidney, lung, brain, striated muscle and large intestine were prepared using this technique. Extracts were also prepared from small intestine of 21-d-old mice and mouse embryos taken at the end of the period of pregnancy. Extracts were also prepared from whole mouse embryos (head included) but with the gastrointestinal tract excised.

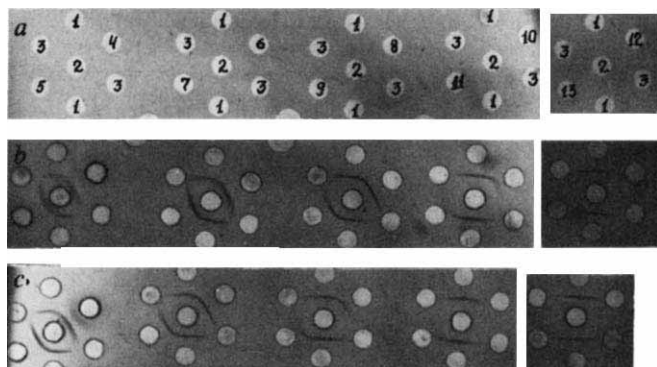


Fig. 1 *a*, Scheme of titration in the microprecipitation in agar. 1–2, test-system (1, antigen, 2, antiserum); 3, physiological solution; 4–13, twofold dilutions of the serum under study from 1:2 to 1:1024. *b*, Titration using serum from control animals (without inoculation of the extracts, day 10). Titre 1:256. *c*, Titration using serum from experimental animals inoculated with 20% extract from small intestine of adult mice (day 10). Titre 1:64.

adults. Using the suppression factor(s) from embryos or adults regulating induction and repression of antigens in normal embryonic cells, it might be possible to affect tumour cells synthesising the same embryonic antigens. These regulating factors were previously unknown, but one is possibly described here. Whether such a factor suppresses AFP synthesis in embryonic liver cells or the multiplication of the cells producing AFP, remains obscure. It seems possible that it would first normalise hepatoma cells, and then inhibit their multiplication. The detection of this suppressing factor seems to confirm the following general approach. Suppression factors affecting the synthesis of a given embryonic antigen must be determined in an organism by starting with that stage of ontogenesis in which their synthesis terminates, and sometimes after this stage. The systematic search for new hormones of embryonic and especially late embryonic and early postnatal periods would be particularly relevant.

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Evidence against clustering of thymine dimers in ultraviolet-irradiated DNA

IRRADIATION of DNA with ultraviolet light causes chemical and physical alterations. From the chemical point of view the main effect of 254 nm UV light is the formation of pyrimidine dimers. The physical consequence of this is a destabilisation of the DNA double helix, presumably by formation of small denatured regions (defects).

According to Shafranovskaya *et al.*¹, the number of defects as determined by the kinetic formaldehyde method is approximately equal to the number of thymine dimers at low fluences of ultraviolet light, but increases much less rapidly than the

yield of dimers at fluences above 10 J m^{-2} . To explain these results they assume that at low fluences every dimer produces a defect, but that at higher fluences dimers are preferentially formed at pre-existing defects after migration of the excitation energy over large distances, up to several thousands of base pairs.

To check this important hypothesis we repeated the experiments of Shafranovskaya *et al.*¹ very carefully taking every precaution to prevent artefacts. Contrary to their findings the concentration of defects did not depart significantly from a linear dependence on ultraviolet fluence. Our experiments therefore contradict the assumption of large distance energy transfer in DNA but of course do not exclude the possibility of energy migration over short distances.

The theory of the kinetic formaldehyde method^{2,3} predicts that denaturation of DNA by the action of formaldehyde follows the equation

$$(-\ln \theta)/t = pv + 2vc + pvt \quad (1)$$

in which c is the initial number of defects per base pair, v the rate of reaction of formaldehyde with base pairs at the boundary of a defect, p the rate for reaction with base pairs in a stable helix and v the average number of base pairs opened up together in the latter process. Under suitably chosen conditions the term pv can be neglected. The degree of helicity θ is defined as

$$\theta = (A_i - A)/(A_i - A_f),$$

where A_i , A and A_f are the initial absorbance, the absorbance at time t and the final absorbance, respectively.

Plotting $(-\ln \theta)/t$ against t gives a straight line with a slope pv and an intercept $2cv$ on extrapolation to $t=0$, and c can be calculated if v is known.

The quotient $(-\ln \theta)/t$ is rather sensitive to errors in A_i . Because the absorbance at the time of mixing of DNA and

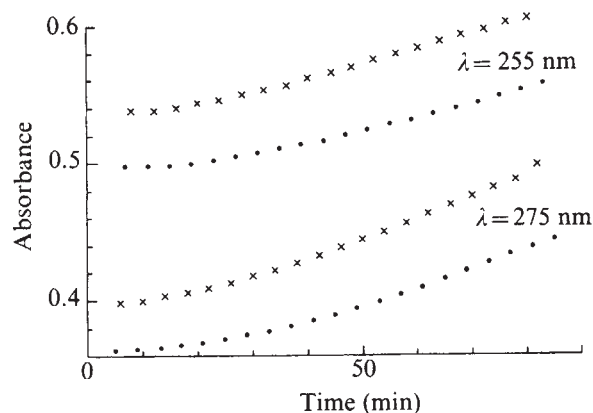


Fig. 1 Change in absorbance of DNA under the action of formaldehyde: ●, non-irradiated DNA; ×, DNA irradiated with 66 J m^{-2} (see legend to Fig. 3). Solvent for DNA was 0.01 M borate buffer, pH 8.7, containing 0.019 M NaCl and 5×10^{-4} M EDTA (ref. 3). Formaldehyde was prepared from paraformaldehyde⁵, kept at 100°C for 5–10 min⁶, neutralised and taken up in concentrated buffer solution to obtain a concentration of 6.7% (w/v) in the same solvent as used for DNA. The final solution was kept at room temperature for at least 2 d to eliminate undesirable drifts in absorbance. Equal weights of DNA and formaldehyde solution were brought into both halves of a two-compartment spectrophotometer cell, equilibrated at 47.5°C for 60 min and then mixed. During the mixing and subsequent measurements the temperature in the cell remained constant within 0.02°C . The transmission of the reaction mixture was measured as a function of time in a Zeiss PMQ II spectrophotometer connected with a digital voltmeter to improve the sensitivity. At 90 min after mixing the temperature of the cell holder was increased to 70°C for 10 min to complete the reaction and then brought down again to determine the final transmission.

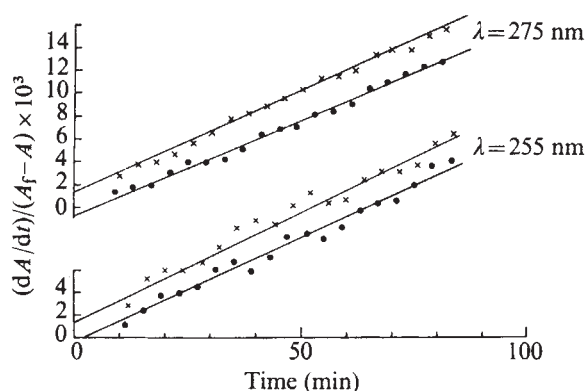


Fig. 2 The kinetics of the reaction of formaldehyde with T2-DNA at 47.5 °C presented as a plot of $(dA/dt)/(A_t - A)$ against t at wavelengths of 255 and 275 nm. ●, non-irradiated DNA; ×, DNA irradiated with 66 J m^{-2} .

formaldehyde cannot be determined very accurately we analysed our data by computer using the equation

$$(dA/dt)/(A_t - A) = 2vc + 2pvt \quad (2)$$

which is in fact the differential equation on which equation (1) is based. The relevant quantity $2vc$ is obtained in the same way as with equation (1), that is, by determining the intercept of a straight line at $t=0$.

The experiments were performed with DNA from the same bacteriophage, T2, as that used by Shafranovskaya *et al.*¹. In this DNA the only pyrimidine dimers produced are thymine dimers. Figure 1 shows the change in absorbance of DNA caused by the action of formaldehyde. All measurements were carried out at 255 and 275 nm, the first wavelength being that of the isosbestic point for the reaction between formaldehyde and denatured DNA. Here the absorbance change is only caused by DNA denaturation, whereas the (larger) change at 275 nm contains a contribution from the formaldehyde reaction itself.

Examples of plots according to equation (2) are given in Fig. 2 for non-irradiated DNA and DNA irradiated with 66 J m^{-2} . The fact that the slopes for both wavelengths do not differ significantly confirms the expectation that reaction with formaldehyde goes hand in hand with denaturation. The wavelength of measurement is therefore not very critical. For reasons as yet unknown, the value of $2vc$ for non-irradiated DNA is slightly negative.

Figure 3 shows that the concentration of defects in DNA increases in proportion to ultraviolet fluences from 0 to 165 J m^{-2} .

These results together with their standard errors are summarised in Table 1. The figures obtained from plots of $(-\ln\theta)/t$ (using extrapolated values of A_i) are also given. Although they are somewhat less reliable, they seem to lead to the same conclusion.

We are unable to indicate exactly why our experiments show

a linear dependence of $2vc$ on ultraviolet fluence, whereas those of Shafranovskaya *et al.* do not. It should be stressed, however, that without careful precautions erroneous and irreproducible results may be obtained because the change in absorbance after mixing may be caused partly by factors other than the reaction of DNA with formaldehyde. First, the temperature must be kept constant within narrow limits. Second, the absorbance of formaldehyde shows a slow drift after a change of temperature, the effect of which is largely eliminated by special pre-equilibration procedures (Fig. 1). Third, it seems that without the pre-equilibration procedures the absorbance of formaldehyde showed a slow drift after mixing with an equal volume of buffer. Fourth, imperfectly cleaned cells sometimes showed a slowly varying blank absorbance.

To check whether the dose-effect curve is linear one does not need to know the value of v . This value was obtained by Shafranovskaya *et al.* by calibrating the kinetic formaldehyde method using single- or double-strand breaks in DNA as defects, the number of which can be determined by ultracentrifugation. Rahn and Stafford⁴ produced evidence that for dimer defects the value of v is much smaller than for break defects. This may explain why at high doses the number of defects seems to be much smaller than the number of dimers, determined by chromatographic techniques. The evidence of Rahn and

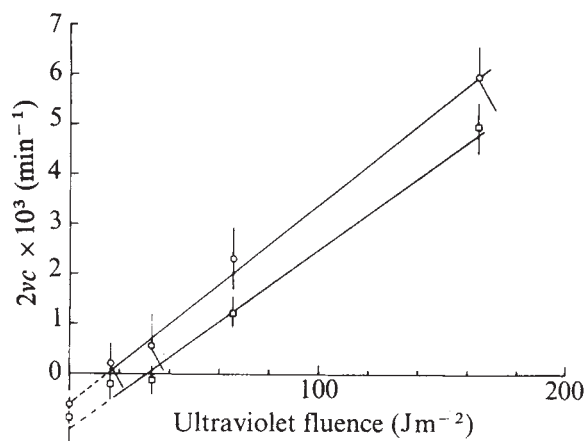


Fig. 3 Relative concentration of defects (value of $2vc$) in T2-DNA as a function of ultraviolet fluence: ○, measurements at 255 nm; □, measurements at 275 nm. Irradiations were carried out with a 15 W low pressure mercury lamp. Solutions (absorbance 0.4) were irradiated horizontally in silica spectrophotometer cells with a light path of 1 cm and containing a small magnetic stirrer. The lamp output was monitored with a Yellow Springs Instrument-Kettering radiometer. The intensity at the position of the cell was $0.65 \text{ J m}^{-2} \text{ s}^{-1}$ and was derived from the inactivation rate of T4 and ϕX174 phage assuming 37% survival at 2.83 and 8.07 J m^{-2} , respectively^{7,8}. The experimental points for irradiated DNA are the weighted averages of two or three measurements. The straight lines were obtained by least squares analysis of all these points. Each of the two points for non-irradiated DNA is the weighted average of seven measurements. Bars represent s.e.

Table 1 Analysis of the measurements*

Calculation number	Wavelength (nm)	$2vc_0 \times 10^3$ (min^{-1})	Intercept $\times 10^3$ (min^{-1})	Irradiated DNA	
				Slope $\times 10^3$ ($\text{min}^{-1} \text{ J}^{-1} \text{ m}^2$)	
A	275	-0.9 ± 0.1	-1.1 ± 0.2	0.036 ± 0.003	
	255	-0.7 ± 0.2	-0.5 ± 0.2	0.040 ± 0.003	
B	275	-0.5 ± 0.2	-0.5 ± 0.9	0.03 ± 0.02	
	255	-0.1 ± 0.2	-0.1 ± 1.2	0.03 ± 0.02	

*Kinetic curves were analysed using one of the formulae (1) and (2) from the text. The data under A were obtained using equation (2), the data under B using equation (1). From the seven measurements for non-irradiated DNA, a weighted average $2vc_0$ was calculated (c_0 : the concentration of defects at zero UV fluence). The values of $2vc$ at ultraviolet fluences between 16 and 170 J m^{-2} were fitted by a straight line (Fig. 3); the intercept and the slope of this line are given. The intercept does not differ significantly from $2vc_0$.

Stafford together with our linear dose-effect relationship makes it very unlikely that the hypothesis of clustering of thymine dimers through long range energy migration is correct.

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Assembly of DNA and protein during replication in HeLa cells

REPLICATION in eukaryotic cells includes not only DNA synthetic processes¹⁻⁴, but also the temporally coordinate⁵⁻⁷ and non-coordinate^{8,9,21} synthesis of chromatin proteins, as well as assembly of these constituents into interphase chromosomes. While much is known about DNA, histone and non-histone chromatin protein biosynthesis, less is known about the time, place and manner of association of proteins with DNA. This communication presents evidence that (1) DNA is replicated with pre-existent chromatin protein still attached; (2) chromatin proteins protect DNA from nuclease digestion in a characteristic manner, unchanged by replicative processes, and (3) addition of new chromatin protein to nascent DNA occurs after a measurable lag period.

Nuclease digestion was used as a probe to examine the extent of DNA-protein associations. Two nucleases are well characterised in chromatin degradations: (i) pancreatic DNase I digests chromatin in a biphasic manner in which DNA is quantitatively degraded, the reaction rate being dependent on the enzyme-substrate ratio^{10,11}; (ii) staphylococcal nuclease ceases digestion after hydrolysis of 45-50% of chromatin DNA (limit digest) irrespective of enzyme load^{11,12,16,22}. Since the two nucleases behave so differently with chromatin, I used each enzyme in parallel digestion studies to ensure that the data were qualitatively in agreement and were not produced by anomalous interplay of either enzyme with the complex deoxyribonucleoprotein substrate.

To study the timing of addition of protein to chromatin, newly synthesised DNA was assayed for nuclease susceptibility. Cells were incubated with ³H-thymidine for periods of 30 s to 32 min, or 12 h for controls (Fig. 1). The kinetics of DNase I digestion of nuclei prepared from each incubation are given in Fig. 1. The most briefly labelled DNAs (30 s-1 min ³H-thymidine) were substantially more accessible to nuclease attack than control chromatin, being intermediate between values for free DNA (>95% degradation) and control chromatin. This enhanced susceptibility was of brief duration, for resistance to digestion increased with the duration of the labelling period, finally to the level of control chromatin within 10-20 min. In Fig. 1b the results are plotted as the amount of each sample digested in 45 min as a function of labelling period, to illustrate more clearly the kinetics of maturation of nuclease resistance. These results are qualitatively the same with both DNase I and staphylococcal nuclease.

That the progression of nuclease-susceptible nascent DNA

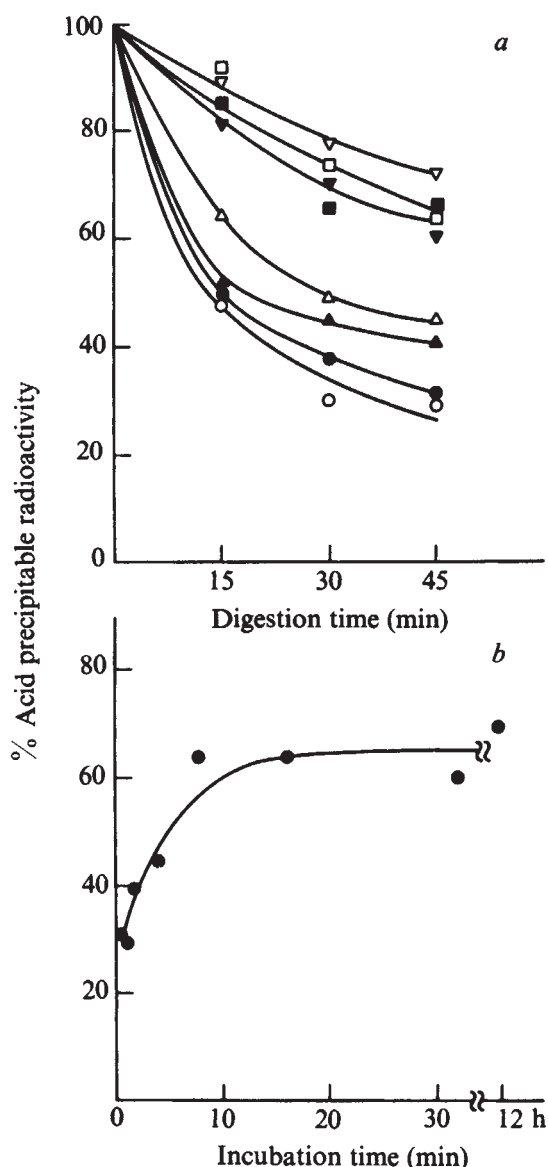


Fig. 1 *a*, Kinetics of DNase I digestion of nuclei labelled for 0.5 (●), 1 (○), 2 (▲), 4 (△), 16 (□), and 32 min (■), and for 12 h (▽) with thymidine. *b*, Nuclease resistance of labelled chromatin after digestion for 45 min. Note different abscissas in *a* and *b*. HeLa S-3 cells, maintained at $2-5 \times 10^5$ ml⁻¹ in Eagle's MEM containing 5% horse serum were concentrated to 2×10^6 ml⁻¹ for labelling. Samples (20 ml) of concentrated cells were incubated with 200, 200, 150, 100, 50, 25 and 10 μ Ci ³H-thymidine (6.7 Ci mmol⁻¹) for 0.5, 1, 2, 4, 8, 16 and 32 min respectively. Control cells were labelled for 12 h at 0.01 μ Ci ml⁻¹. Labelling was terminated by immersion of the tube in ice and addition of 2 volumes of RSB (10 mM Tris-HCl, pH 7.6, 1.5 mM MgCl₂, 10 mM NaCl) containing 0.2% sodium azide. The cells were collected by centrifugation at 1,800g (maximum) for 1 min. The pellet was resuspended and washed again in 20 ml RSB. After resuspension in RSB the cells were swollen for 10 min and then 0.1 volume 10% Triton X-100 was added. The cells were swirled on a Vortex mixer for 30 s and then centrifuged at 1,800g for 1 min. The nuclei were washed twice more in RSB. The final nuclear suspension in RSB was adjusted to a concentration of 3.0 A_{280} units ml⁻¹, as measured in 1% sodium dodecyl sulphate. Samples (0.1 ml) of the nuclear suspension were added to tubes, followed by 0.8 ml RSB. Then, 5 μ g DNase I (Worthington) in 0.1 ml RSB was added and, after mixing, the tubes were placed in a 37 °C water bath. At the indicated times, reactions were stopped by placing tubes in ice and adding 2 ml 10% trichloroacetic acid. Samples (100 μ g) each of bovine serum albumin and calf thymus DNA were added to each tube and the precipitates were collected on Whatman GF/C filters. To each filter, 0.2 ml of a 9:1 NCS (Amersham/Searle)-water mixture was added, followed by 10 ml of 0.4% PPO in toluene. Vials were incubated for 12-16 h at 37 °C before counting in a Beckman LS-250 liquid scintillation spectrometer.

to the relatively nuclease-resistant control chromatin represented a true precursor-product relationship, was demonstrated by labelling nascent DNA by brief incubation with isotope, and following the fate of the incorporated radioactivity after removal of isotope (Fig. 2). Cells were incubated with ^3H -thymidine for 90 s and, after removal of isotope, samples were collected at intervals for 30 min. The nuclease resistance of the 90 s labelled material was again 50% that of controls. Resistance of the radioactive DNA to digestion increased progressively with time to reach controls after 10–15 min of further incubation. That the digestibility of the first sample taken after labelling was already less than the 90 s sample, reflects the 5 min required for the washing procedure. There was no evidence that newly synthesised DNA was protected from nucleases,

Fig. 2 Digestibility of briefly labelled chromatin DNA after removal of label. Cells, as described in the legend to Fig. 1, were incubated with $10\ \mu\text{Ci}\ ^3\text{H}$ -thymidine ml^{-1} for 90 s. To terminate labelling, non-radioactive thymidine was added to $50\ \mu\text{g}\ \text{ml}^{-1}$. A sample was removed for the initial sample and the cells were centrifuged at $1,800g$ (maximum) for 1 min. Cells were resuspended in 37°C medium containing $50\ \mu\text{g}$ thymidine ml^{-1} , centrifuged again, and finally resuspended in fresh thymidine-containing medium for the times indicated. Samples were collected and processed at the times indicated as described for Fig. 1. ●, ^3H -thymidine incubation for 90 s; ○, zero time after isotope removal; ▲, further incubation for 2 min; △, 4 min; ■, 16 min; □, 32 min; ▽, one generation label.

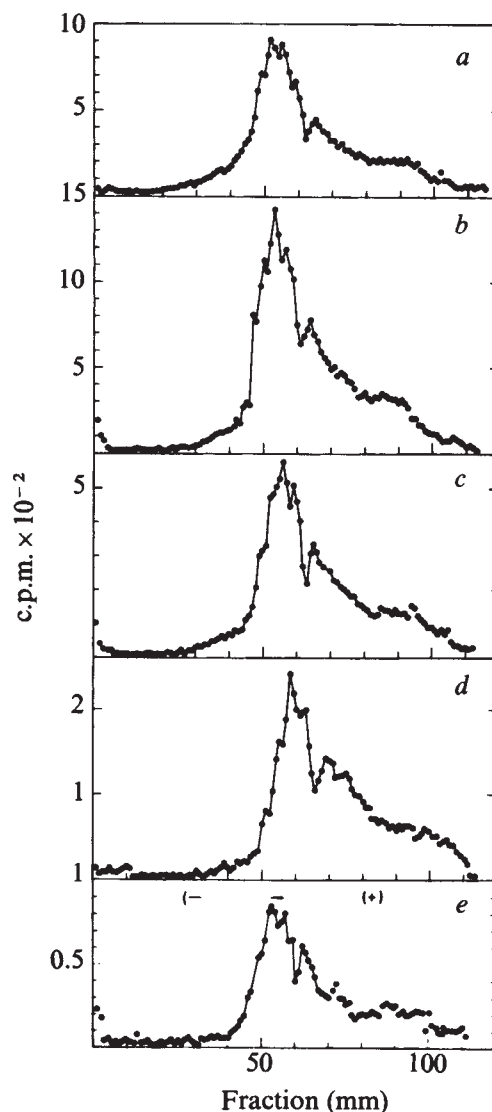
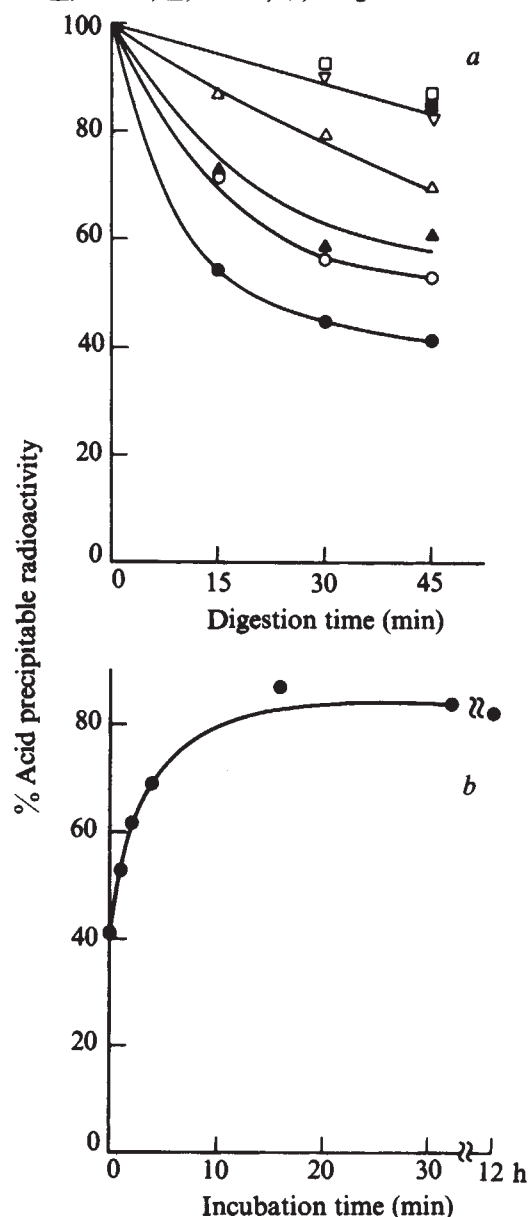


Fig. 3 Polyacrylamide gel electrophoresis of nuclease-resistant DNA fragments from staphylococcal nuclease limit digests. *a*, one generation label; *b*, 10 min; *c*, 4 min; *d*, 90 s; *e*, 45 s. Cells were incubated with $10\ \mu\text{Ci}\ \text{ml}^{-1}$ (*b-e*) or for 16 h with $0.01\ \mu\text{Ci}\ \text{ml}^{-1}$ ^3H -thymidine (*a*), respectively. Nuclear digestions were performed in 5 mM phosphate pH 7.6, $2.5 \times 10^{-6}\ \text{M}\ \text{Ca}^{2+}$ with $1\ \mu\text{g}$ of staphylococcal nuclease. After 90 min precipitates were collected by centrifugation at $17,000g$ for 10 min. The precipitates were dissolved in 1% SDS, 0.1 M NaCl, 1 mM EDTA, 10 mM Tris, pH 7.6, extracted three times with chloroform isoamyl alcohol (24:1), and precipitated with 2 volumes ethanol at -20°C for 16 h. DNA was dissolved in electrophoresis buffer containing 6 M urea. The samples were applied to $0.6 \times 10\text{-cm}$ 6% acrylamide gels (20:1 acrylamide-*bis* acrylamide) and were subjected to electrophoresis in buffer containing 0.089 mM Tris, pH 7.6, 0.90 M boric acid and 6.5 mM disodium EDTA¹⁷ for 2 h at 2 mA per gel. Gels were scanned at 260 nm in a Beckman Acta III recording spectrophotometer equipped with a linear transport device. The gels were then fractionated into 1-mm segments with a Mickle Gel Slicer (Brinkmann Instruments). Gel slices were incubated in 0.5 ml NCS (Amersham/Searle)-water (9:1) at 50°C , 12 h. Scintillation fluid was then added, and after 6 h at 37°C counting was carried out as in Fig. 1.

relative to controls, in contrast to a report on nuclease susceptibility of nascent DNA in chick erythrocyte chromatin¹⁵. The time for unidirectional synthesis of replicon-sized units ($3.5\text{--}15\ \mu\text{m}$)¹ of DNA at chain elongation rates of $0.8\text{--}2\ \mu\text{m}\ \text{min}^{-1}$ (refs 1, 3 and 18–20) is about 5–15 min. Thus the period of 10–15 min observed for maturation of nuclease resistance, interpreted to represent chromatin protein addition, corresponds with that for completion of replicons.

Staphylococcal nuclease digestions produce a true limit digest of chromatin^{11,21,22} and the resistant DNA can be resolved in polyacrylamide gels as a series of fragments of discrete sizes¹⁶. By this method, the size classes of resistant DNA from control chromatin were compared with patterns from briefly labelled chromatin as described in Figs 1 and 2 (Fig. 3). The patterns of nuclease-resistant DNA fragments derived from cells labelled for 45 s, 90 s, 2 min, 4 min, 10 min and 17 h were all the same. Recoveries from gels averaged 80-85% in all cases.

These results can be interpreted in the form of a simplified model. Chromatin is represented as DNA complexed with discrete protein oligomers^{13,14}, possibly of different, but a limited variety of sizes. As DNA is replicated, these protein oligomers segregate with the daughter chromosomes such that the newly synthesised DNA has half the protein content of mature chromatin. As replicon-sized DNA fragments are synthesised (about 10-20 min), new protein is added and nuclease resistance increases to that of parental chromatin. Because the size of nuclease-resistant DNA fragments is identical at all times, I propose that chromatin proteins (histones) associate with the new DNA in oligomeric form. Thus, all chromatin, regardless of the state of maturation and nuclease resistance, yields the same size classes of resistant DNA fragments.

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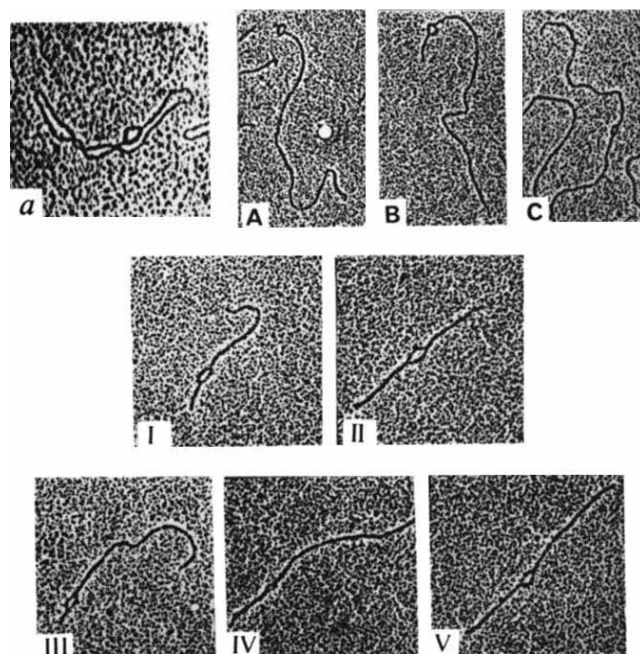


Fig. 1 Electron micrographs of polyoma DNA molecules carrying a denaturation loop induced by gene 32 protein. *a*, Closed polyoma DNA molecule carrying a denaturation loop. A, B and C, *EcoRI* cleaved polyoma DNA molecules carrying denaturation loop induced by gene 32 protein at three different sites. I and II, 43% fragment from *HinIII* digestion of Py DNA denatured by gene 32 protein with a denaturation loop at one of two sites. III-V, 57% fragment from *HinIII* digestion of polyoma DNA denatured by gene 32 protein with a denaturation loop at one of three sites. Gene 32 protein was purified from *E. coli* cells infected with an amber mutant of T4 bacteriophage (lysosome-) obtained from the New England Enzyme Centre, Massachusetts, using the method of Alberts and Frey¹. Polyoma DNA was labelled with ³H-thymidine and extracted from cells infected with A-2 strain of large plaque virus at low multiplicities of infection² by the method of Hirt³ followed by phenol extraction, equilibrium centrifugation in CsCl-ethidium bromide, and purification by neutral sucrose density gradient centrifugation. Complexes of polyoma DNA (form I) with T4 gene 32 protein were prepared according to Delius *et al.*⁴. After fixation in glutaraldehyde the sample was dialysed against 0.01 M Tris-HCl, pH 7.4, containing 0.01 M NaCl and 1 mM EDTA at 4 °C, and concentrated. Conditions were then adjusted and digestion carried out with endonuclease *EcoRI* and *HinIII* as described by Griffin *et al.*⁵. The preparation of samples for electron microscopy was carried out as described by Davis *et al.*⁶. An internal length standard of PM2 DNA (taken as 9,900 nucleotide pairs) was used. Measurements were made using a Hewlett Packard digitiser interfaced with a 9820A calculator.

Denaturation of polyoma DNA by phage T4 gene 32 protein

POLYOMA virus is a small oncogenic virus containing a circular DNA, the identification and location of defined regions of which provides physical reference points which are important for correlation in genetic and biochemical studies. The protein coded by gene 32 of bacteriophage T4 binds tightly to single-stranded DNA and RNA and facilitates denaturation and renaturation of double-stranded DNA^{1,2}. Using glutaraldehyde fixation gene 32 protein denaturation of double-stranded DNA can be observed using electron microscopy at physiological temperatures, and denaturation maps are produced which closely resemble those obtained with heat and alkaline denaturation². Use of gene 32 protein in binding experiments with circular supercoiled DNA molecules from polyoma virus and simian

virus 40 (SV40) has already shown that only one denaturation loop is formed per molecule²⁻⁴ reflecting possible structural constraints in the DNA. The characterisation and mapping of the preferred denaturation site(s)⁴ is also of interest since these putative A+T-rich region(s) are likely to be related to the binding sites of enzymes using DNA as a template. In the present study the use of two different specific restriction enzymes (endonucleases) has enabled us to identify five regions of polyoma DNA which are preferentially denatured by phage T4 gene 32 protein, and the determination of their precise locations on the circular viral DNA.

When polyoma DNA was incubated with gene 32 protein (Fig. 1*a*), only one denaturation region was seen per molecule of DNA (with one exception) and the size of the denaturation loop formed was about 5% (or 260 base pairs) of the full length of the molecule. The presence of gene 32 protein increased the length of the DNA molecule by about 4%.

When circular polyoma DNA molecules containing a

Table 1 Possible locations and frequencies of the individual denaturation sites induced by gene 32, measured on the two fragments produced by digestion of polyoma DNA using endonuclease *Hin*III

<i>Hin</i> III fragment, gene 32 protein complex	Possible locations	s.d.	Frequency
I	0.08* or 0.36	± .03	11%
II	0.20* or 0.24*	± .02	33%
III	0.03 or 0.47*	± .02	14%
IV	0.12 or 0.44*	± .02	27%
V	0.20* or 0.36	± .01	14%

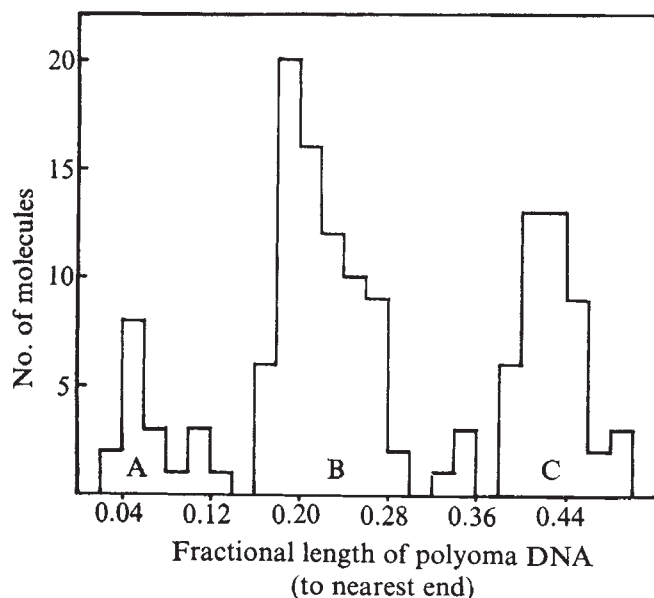
Measurements on *Hin*III fragments were converted to percentage total genome, referred to the *Eco*RI cleavage site and mapped to the nearest end of a full length linear molecule. The two possible locations for each denaturation loop, depending on the orientation of the fragment, are shown with the most probable location asterisked.

I and II correspond to 43% *Hin*III fragment species (Fig. 1); III, IV, and V correspond to 57% *Hin*III fragment species (Fig. 1).

denaturation loop were digested with restriction enzyme *Eco*RI (which cuts polyoma DNA at one unique site^{7,9}) three types of molecule were observed, each with the denaturation loop at a particular site (Fig. 1A, B and C). Figure 2 shows that the three preferred sites for denaturation are located, respectively, at 0.07, 0.23 and 0.42 fractional length from the nearest end of the molecule and with frequencies of 12%, 52% and 36%. These observations are in agreement with those reported by Yaniv *et al.*⁴.

The unambiguous location of these sites on the circular DNA molecule, however, could only be achieved using another specific restriction enzyme capable of distinguishing between the two ends of the linear molecule cleaved by *Eco*RI. This was accomplished using endonuclease *Hin*III which cuts polyoma DNA at two sites⁷ generating two fragments, one 57±2% and the other 43±2% of the genome (Fig. 3). When polyoma DNA gene 32 protein complexes were digested using this enzyme, the two fragments were obtained, one about 43% of the genome with a denaturation loop at one of two sites, and the other about 57% of the genome with a denaturation loop at one of three sites (Fig. 1, I-V). Table 1 shows that four of the sites found on the *Hin*III fragments must be located in positions equidistant from the *Eco*RI site and thus together comprise two

Fig. 2 Location of denaturation sites (A, B, C) induced by gene 32 protein on *Eco*RI cleaved polyoma DNA gene 32 complexes (expressed as fractional length to the nearest end). Measurements were carried out to the midpoint of the denaturation loop. Average locations were: A, 0.07 ± 0.03; B, 0.23 ± 0.06; C, 0.42 ± 0.07. Relative frequencies were: A, 12%; B, 52%; C, 36%.



of the sites found on the *Eco*RI linears. As we knew the location of the two fragments on the circular map⁷, we determined the absolute positions of the loops by comparing the *Hin*III data with the distribution of loops on the *Eco*RI linears.

Taking the 43% fragment first, each of the sites has two possible map locations, depending on which way the fragment is oriented (Fig. 4b and c). Because one of the loops is located at about the midpoint of the fragment either at 0.20 or 0.24 on the map, however, this immediately fixes the position of this denaturation region (II in Figs 1 and 4) as corresponding to B at 0.23 from the *Eco*RI linear map (also shown for comparison in Fig. 4). The other denatura-

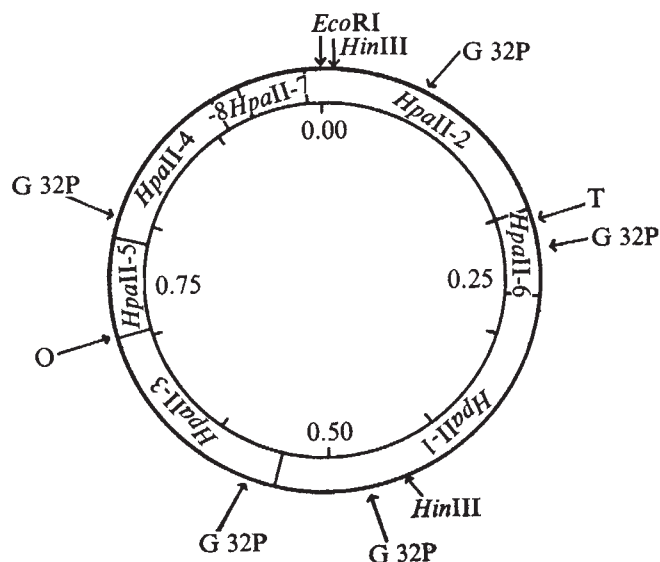


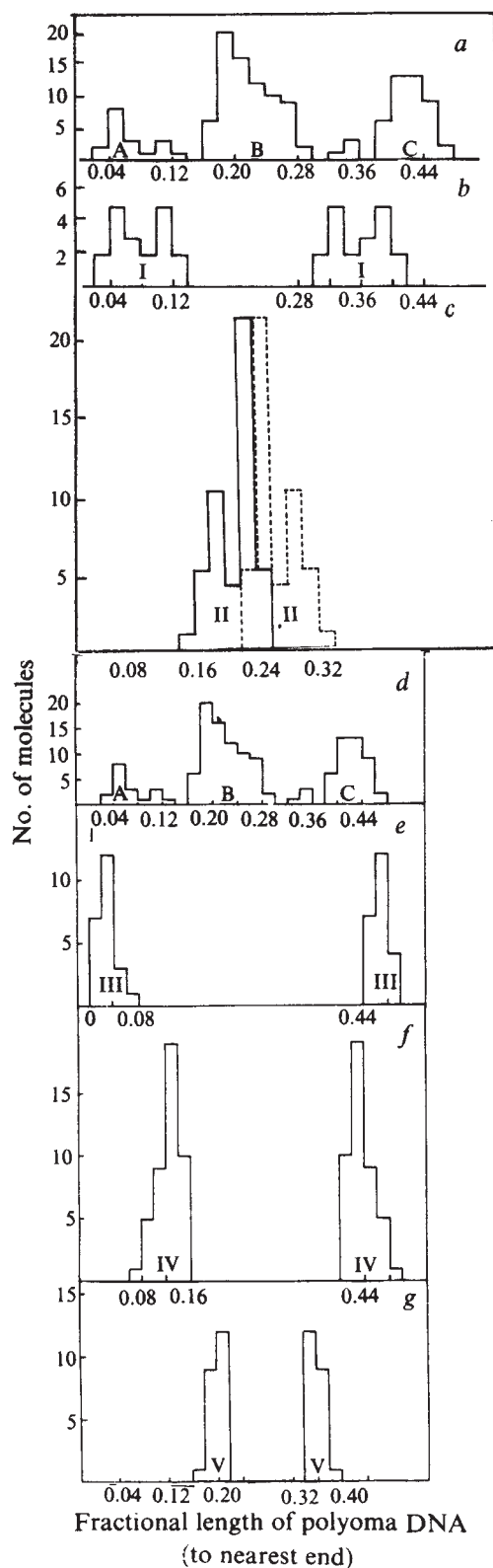
Fig. 3 Polyoma DNA map showing cleavage sites for enzymes *Eco*RI, *Hin*III and *Hpa*II (from Griffin *et al.*⁷), and gene 32 protein (G32P) denaturation regions referred to in the text. O and T are origin and termination of DNA synthesis respectively. Full length is 1 unit.

tion region observed on the 43% fragment could occupy either position 0.08 or 0.36 (Fig. 4b). Comparison with the *Eco*RI linear map shows that there is a denaturation region which could correspond to the 0.08 position but none at 0.36. This observation identifies site I (Figs 1 and 4) as being A on the *Eco*RI map. On comparing the frequencies of these sites (I and II) with the *Eco*RI frequencies, we found that whereas the frequency of denaturation site I on the small fragments agrees with that observed for site A on the *Eco*RI linears (11% as opposed to 12%) site II only accounts for 33% of the denaturation sites observed (as opposed to 52% incidence of denaturation site B on *Eco*RI). This would therefore imply that site B is a 'double' site, that is, one made up of two sites, equidistant from the *Eco*RI cleavage site. This interpretation would predict that another denaturation loop would be found on the 57% fragment, corresponding to map position about 0.77 (Fig. 3); site V fits these requirements (Fig. 4g). Its frequency of 14% brings the overall frequency of II+V to 47% in reasonable agreement with the 52% frequency observed for the B loop on the *Eco*RI linears. The alter-

Table 2 Frequencies and locations of the various denaturation sites measured on *Hin* III fragments compared with distribution on *Eco*RI linears

<i>Eco</i> RI		<i>Hin</i> III	
Location	Frequency	Location	Frequency
0.07 ± .03	12%	0.08 ± .03	11%
0.23 ± .06	52%	0.23 ± .03	47%
0.42 ± .07	36%	0.44 ± .04	41%

Fig. 4 Alternative map locations of denaturation sites induced by gene 32 protein on the *Hin*III fragments expressed as the fractional genome length from the nearest *Eco*RI end. *a-c*, 43% *Hin*III fragment. *a*, Distribution of denaturation loops on *Eco*RI-cleaved polyoma DNA-gene 32 protein complexes for comparison. *b*, *Hin*III 43% fragment with denaturation loop (I) shown in Fig. 1. *c*, *Hin*III 43% fragment with denaturation loop (II) shown in Fig. 1. *d-g*, 57% *Hin*III fragment. *d*, Distribution of denaturation loops on *Eco*RI linears as in (*a*)



for comparison. *e*, *Hin*III 57% fragment carrying denaturation loop (III) as shown in Fig. 1. Note that the histogram on the right is not the 'mirror image' of the one on the left, as is the case in (*f*) and (*g*). This is a result of the second possible location extending to either side of the midpoint (0.5), and being expressed as fractional genome length from the nearest *Eco*RI end. *f*, *Hin*III 57% fragment carrying denaturation loop (IV) as shown in Fig. 1. *g*, *Hin*III 57% fragment carrying denaturation loop (V) as shown in Fig. 1.

native position for denaturation site V at 0.36 does not correspond to a site found on the *Eco*RI map.

For the two other denaturation sites observed on the large *Hin*III fragment (57%) the same approach was followed. Figure 4*e* and *f* shows that sites III and IV must occupy approximately equidistant positions in relation to the *Eco*RI cleavage site: one at map position 0.47 and the other at position 0.56 together accounting for site C at 0.43 on the *Eco*RI map (Fig. 4*d*). When the frequencies observed for these two locations are added a total of 41% is obtained in reasonable agreement with that observed for C (36%). Finally, data (not shown) from digestion of polyoma DNA gene 32 protein complexes with another enzyme—*Hpa*II—generating eight fragments⁷, also confirms the presence of five easily denaturable regions.

In summary, there seems to be on polyoma DNA five sites which are readily denatured by gene 32 protein with map positions (Fig. 3) of approximately 0.08, 0.23, 0.47, 0.56 and 0.80 and frequencies of 11%, 33%, 14%, 27% and 14%, respectively. The *Hin*III data when expressed as percentage total genome and referred to the nearest end compares well with the *Eco*RI linear map (Table 2). Note that the preferred sites for denaturation by gene 32 protein correspond to *Hpa*II fragments 2, 6, 1, 3 and 4 (Fig. 3) and that 2 and 6 were the fragments found by Giffin *et al.*⁷ as having the highest A+T/G+C ratio. The origin of DNA replication which has been mapped at 0.71 (refs 7 and 10) does not seem to be associated with an A+T-rich region whereas termination of DNA synthesis at 0.21 (Fig. 3) is closely associated with the most frequent denaturation site at 0.23. The present work using two different restriction enzymes overcomes the previous difficulties in trying to locate A+T-rich regions in circular viral DNA molecules^{11,12}. The functional significance of each of the A+T-rich regions described in this study remains to be established.

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Note added in proof: Lescure and Yaniv (personal communication) also have evidence suggesting the presence of a gene 32 protein induced denaturation site mapping at ~0.80.

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Proximity of deoxyadenylic-thymidylic acid clusters to origin of DNA replication in *E. coli* 15T⁻ cells

CLUSTERS of deoxyadenylic-thymidylic (dA-T) and deoxycytidylic-deoxyguanylic acid (dC-dG) residues have been detected in DNAs from many sources^{1,2}. The purine or pyrimidine clusters in denatured DNA can hybridise with complementary polyribonucleotides and the greater density of the complexes means that they can be separated from unreacted DNA by isopycnic centrifugation in CsCl or Cs₂SO₄ (refs 1 and 2). These clusters are estimated to be 10-100 residues long and are distributed throughout DNA at intervals of 10⁵-10⁶ daltons (ref. 3). The dC-dG clusters have been implicated in the regulation of transcription in viruses⁴⁻⁸, bacteria^{1,9} and in nuclei (P. K. Bhattacharya and H.K., unpublished) from eukaryotic cells (for reviews see refs 3 and 10). Less is known about the function of clusters of dA-T residues which are as common in most DNAs¹. Although in adenoviruses the presence of dA-T clusters has been correlated with the oncogenic potential¹¹ and Borst and Ruttenberg¹² found runs of dT on the transcribed strand of mitochondrial DNA, no further efforts have been made to associate regulatory or other functions with the presence of these clusters. We now report observations which suggest that clusters of dA-T may be involved in the initiation of DNA synthesis. The rationale for our investigation was that if such clusters were detected at or near the replication origin, it would be consistent with the proposition that they function in the initiation of DNA replication.

If a synchronised culture of *Escherichia coli* is pulsed with ³H-thymidine when DNA replication resumes and subsequently chased with cold thymidine, newly replicated DNA near the origin is labelled whereas that farther from the origin and all of the unreplicated portion of the DNA is unlabelled (Fig. 1). Since replication in *E. coli* is bidirectional^{13,14}, labelled regions on both sides of the origin (0) would be anticipated (thick lines).

Fig. 1 Schematic representation of the experimental design. O, point of origin (starting point of DNA replication); I and II, two dA-T clusters of opposite polarity; the thick and the thin lines denote the labelled and unlabelled portions of cellular DNA, respectively; strings of Us, poly(U); the two arrows show the positions of breaks in DNA during shearing.

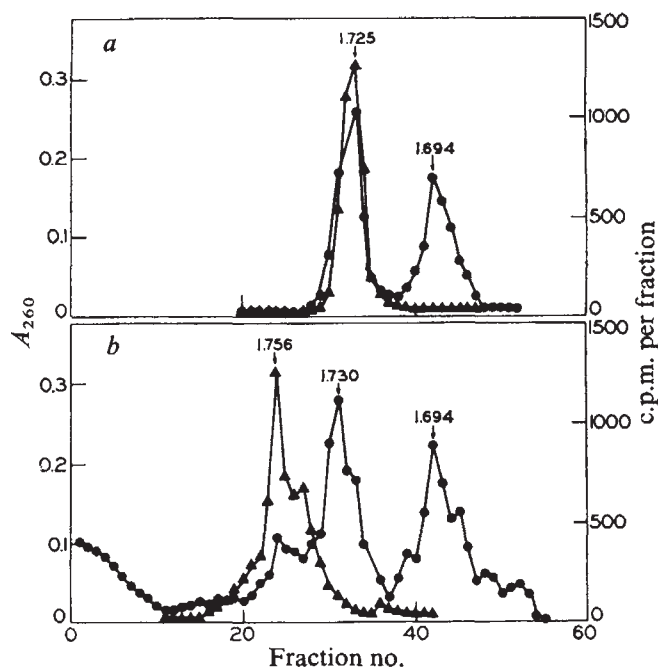
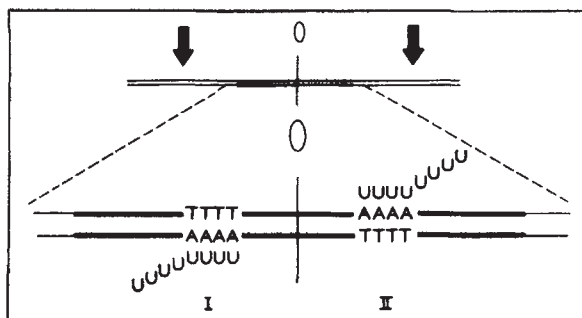


Fig. 2 Distribution in CsCl gradients of pulse labelled, sheared and denatured *E. coli* 15T⁻ DNA (200 µg per sample) in the absence of any polyribonucleotide (a) and in the presence of 500 µg of poly(U) (b). DNA was extracted from synchronised, ³H-thymidine-labelled cells, sheared, denatured and reacted with poly(U) as described in the text. The samples were centrifuged together with 120 µl of marker native *Cytophaga johnsonii* DNA (density in CsCl 1.694 g ml⁻¹, see ref. 1) in a Spinco SW 50.1 rotor, 38,000 r.p.m. at 8 °C. Fractions were collected at the end of the 48-h run. ●, Absorbance at 260 nm; ▲, radioactivity.

When this DNA is sheared into fragments shorter than the average distance between two adjacent clusters (breaks depicted by arrows in Fig. 1), denatured and reacted with a complementary ribopolymer, polyuridylic acid (poly(U)), fragments containing dA runs should band at the density higher than that of DNA alone (density of RNA is close to 1.9 g ml⁻¹ in CsCl¹⁴). Fragments lacking dA runs should band at the normal density of denatured *E. coli* DNA (1.725 g ml⁻¹ in CsCl^{1,15}). (The unsheared DNA will band at the higher density since dA-T clusters are distributed evenly throughout *E. coli* DNA¹.) Thus, if a dA-T cluster (I in Fig. 1) is located between the two breaks, either inside or outside the labelled region, half of the labelled DNA (that strand which contains the dA run) should become heavier than the bulk of the unlabelled DNA in the presence of poly(U). If two dA-T clusters of opposite polarity (that is, with the dA segments present on opposite strands) are located close to 0, as are clusters I and II in Fig. 1, all the radioactive DNA will hybridise with poly(U) and become more dense than the bulk of the DNA, which is defined as the bulk of the ultraviolet-absorbing material. Thus, the experiment tells whether runs of dA are located close to the origin and whether they occur on one or both strands in that region.

Similarly, the occurrence of dC-dG clusters near the origin could be detected if polyriboguanilic acid (poly(G)) or the heteropolymer of guanylic and uridylic acids (poly(U,G)), is substituted for poly(U). Poly(U,G) has the same specificity as poly(G) but fewer technical problems are associated with its use^{3,9,16}.

A culture of *E. coli* 15T⁻ (auxotrophic for thymine, arginine, methionine and tryptophan) was synchronised by the amino acid starvation technique¹⁷. The culture was supplemented with ³H-thymidine (0.65 µCi, specific activity 1.56 Ci mmol⁻¹), immediately resupplied with the required amino acids and incubated for 15 s at 25 °C. Unlabelled thymidine was then added up to the concentration of 20 µg ml⁻¹ and incubation continued for a further 2 min at 25 °C. The cells were chilled,

collected and lysed by standard techniques. DNA was extracted¹⁸ and divided into two portions, one of which was sheared by forcing the solution four times through a 27-gauge needle. Both DNA samples were then heat denatured¹ and each was divided further into three portions. Poly(U) was added to one portion and poly(U,G) to the second. The third contained no synthetic polynucleotide. A saturated solution of CsCl was added, the samples were centrifuged until equilibrium and the distributions of radioactive and ultraviolet-absorbing materials in the gradient were determined³. Some of the results are shown in Fig 2. Denatured DNA, not exposed to polyribonucleotide, banded in CsCl at a density close to 1.72 g ml⁻¹ and the radioactive and ultraviolet-absorbing fractions practically overlapped (Fig. 2a). In the presence of poly(U) (Fig. 2b) the DNA came to equilibrium at a higher density and the ultraviolet-absorbing material was distributed over a wider range than in the control. All the radioactive DNA banded as a rather homogeneous peak well below the bulk of the DNA.

In several parallel experiments (not shown) the distribution patterns of the radioactive DNA and of the bulk of the unlabelled DNA in the gradient did not differ when: (1) the DNA was not sheared; (2) the pulse was longer than 15 s; (3) the pulse was begun more than 15 s after addition of the required amino acids; (4) either sheared or unsheared DNA was exposed to poly(U,G) instead of poly(U) and (5) DNA was obtained from non-synchronised cultures labelled as described.

The experiments summarised suggest that dA-T clusters (but not clusters of dC-dG) are located close to the replication origin in *E. coli* 15T⁻. Transposition by poly(U) of almost all the radioactive DNA from the density occupied by the bulk of cellular DNA suggests that at least two dA-T clusters of opposite polarity are located close to this point. The presence of a single heteropolymer composed of a run of dA residues followed by a run of Ts on the same strand, with complementary bases on the opposite strand, however, would account for the observed phenomena equally well.

We do not know how close these clusters are to the origin. They may be immediately adjacent to it, forming part of the sequence used by DNA polymerases to initiate DNA replication; or they may be as far as 500–700 nucleotide pairs away, half the length of the average DNA sheared in our conditions (unpublished observations). The latter estimate of this distance is probably too high in view of the relative homogeneity of the poly(U)-transposed radioactive peak (Fig. 2b). The fact that the dA-T clusters are also distributed throughout the DNA¹ may be related to repeated reinitiation of DNA synthesis¹⁹.

The experiments described here do not suggest the precise role (if any) played by the dA-T clusters in DNA synthesis, although their location next to the starting point of replication may indicate some vital function. No information is available on the proximity of such clusters to the origin of DNA replication in other prokaryotic and eukaryotic cells. Identification of such structures in the vicinity of this site in DNA from other organisms would make a stronger case for their involvement in regulation of the replication process.

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Variable redundancy in RNA transcripts isolated in S and G₂ phase of the cell cycle of *Physarum*

GENOMES of eukaryotes contain variable proportions of repeated base sequences of mostly unknown function¹. It has been shown that nuclear RNA of sea urchin², rat³ and *Dictyostelium*⁴ also contains repetitive sequence elements, whereas polysomal messenger RNA (mRNA) does not. If mRNA is contained in the heterogeneous nuclear RNA, these sequence homology experiments suggest a selective processing of 3'-fractions of nuclear poly(A) RNA with unique base sequences. Most mRNA fractions and individual mRNAs so far studied^{2,4–7} hybridise with single-copy DNA, with the exception of *Xenopus* embryo mRNA which contains both unique and moderately repeated sequences⁸. Functions for the repeated base sequences in RNA are not known and could involve regulation of transcription, processing, or translation, as well as copying of some sequences significant for DNA replication and recombination^{1,9}. We have now used the naturally synchronous mitotic cycle of *Physarum*¹⁰ to investigate whether there are changes in the degree of repeated sequences in RNA transcribed in S phase and G₂ phase.

We isolated poly(A)-rich RNA by affinity chromatography¹¹ from nuclei¹² and polysomes¹³, and sedimented these molecules in sucrose gradients. The nuclear preparations were heterogeneous (Fig. 1a). They contained RNA fractions ranging from 4S to 30S, but no larger RNA molecules, whereas most of the poly(A)-rich RNA from polysomes sedimented with about 8S–15S (Fig. 1b). The RNA from nuclear preparations was then divided into RNA molecules of the size of polysomal RNA (Fig. 1a, fractions 13–22) and RNA molecules larger than polysomal RNA (Fig. 1a, fractions 1–12). Then the reassociation kinetics of these RNA molecules with DNA at an excess DNA concentration was determined¹⁴. We found that small molecules from nuclei and the RNA obtained from polysomes hybridised mainly with single-copy DNA (Fig. 1c) as judged from previous *C₀t* curves of nuclear DNA from *Physarum*¹⁵. A significant proportion of the heavy nuclear poly(A)-rich RNA had, however, hybridised at much lower *C₀t* values (Fig. 1c). To rule out hybridisation artefacts in comparing large and small RNA molecules, the heavy fraction of nuclear RNA was sonicated to about the size of polysomal RNA. The hybridisation kinetics of the shortened RNA molecules was indistinguishable from that of large RNA molecules (Fig. 1c).

From these observations we deduce that in *Physarum*, as in *Dictyostelium*⁴, and perhaps in most higher cells¹, a fraction of nuclear RNA contains repeated base sequences which are lost in processing from the nucleus to the cytoplasm.

In the next experiment (and others not shown) we analysed the reassociation kinetics of nuclear poly(A)-rich RNA prepared in S phase or G₂ phase and found that the sample from G₂ phase contained a significantly higher proportion (35%) of redundant

Fig. 1 Sucrose gradient profiles and hybridisation kinetics of poly(A)-rich RNA from *Physarum polycephalum*. Plasmodia were grown¹⁸ and labelled¹¹ as described previously, for 1 h with 100 $\mu\text{Ci ml}^{-1}$ ^3H -uridine. Nuclei¹² and polysomes¹³ from *Physarum* were prepared using published methods. RNA was extracted with a phenol procedure adapted for *Physarum*¹⁹⁻²¹, utilising trisopropyl-naphtalensulphonate for lysis and diethyl-pyrocabonate as RNase inhibitor. Poly(A) RNA from nuclear and polysomal RNA was isolated by affinity chromatography on poly(dT) cellulose as reported previously^{11,22} and fractionated on linear 5–20% sucrose gradients as published¹¹. For hybridisation experiments aliquots of poly(A) RNA of about 10,000 c.p.m. were incubated with 1 mg ml^{-1} DNA in $2\times\text{SSC}$, 50 mM Tris, pH 7.2, 0.1% SDS in 50% formamide^{3,14,23}. Nuclear DNA was prepared, purified, sheared and heat denatured as described previously¹⁸. At appropriate C_0t values the percentage of hybridisation was computed for each point from the radioactivity resistant to a mixture of RNase A and T1. At a C_0t value of 10^3 approximately 65–80% (700–900 c.p.m.) of the input RNA had hybridised. *a*, Profile of nuclear poly(A) RNA; *b*, profile of polysomal poly(A) RNA; *c*, hybridisation kinetics of poly(A) RNA from *Physarum*. $\blacktriangle$, Polysomal RNA; $\circ$, large nuclear RNA molecules; $\triangle$, large nuclear RNA, sonicated; $\bullet$, small nuclear RNA molecules.

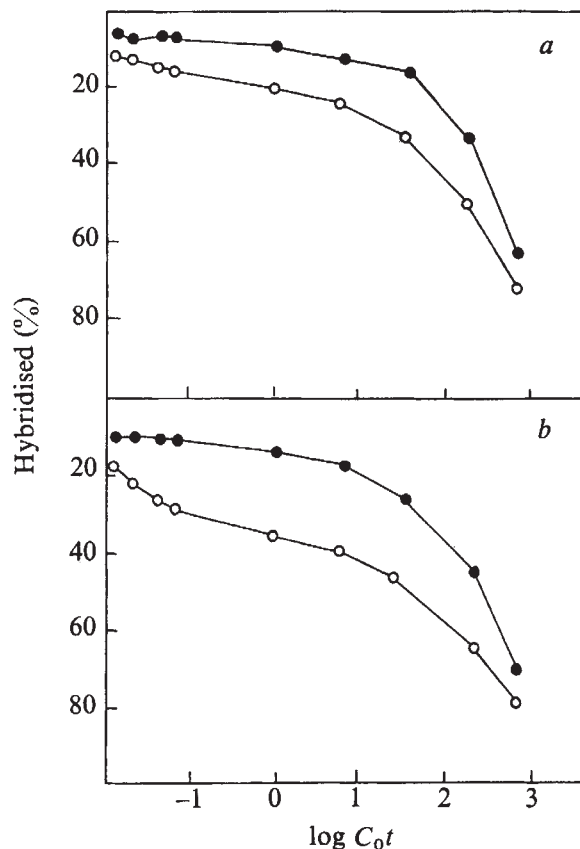
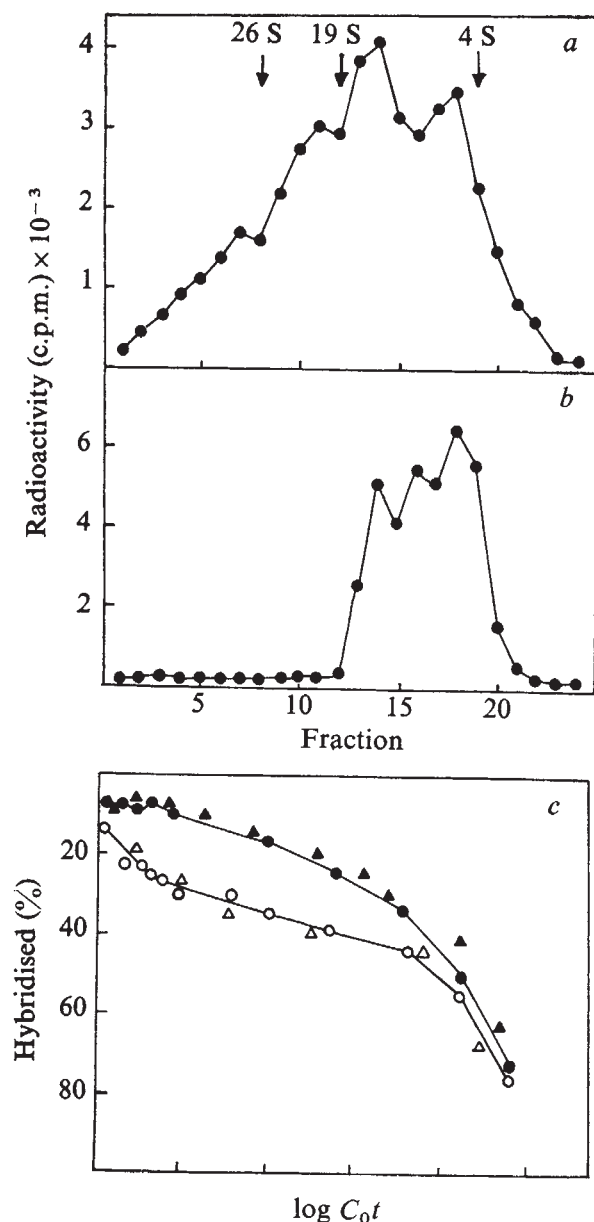


Fig. 2 Hybridisation kinetics of nuclear and polysomal poly(A) RNA prepared in S phase and G_2 phase. *Plasmodia* were labelled for 1 h in S phase (5–65 min post mitosis) and G_2 phase (4–5 h post mitosis). Preparation of subcellular poly(A) RNA fractions and hybridisation procedures were as described for Fig. 1. *a*, S phase of the cell cycle; $\bullet$, polysomal poly(A) RNA; $\circ$, nuclear poly(A) RNA; *b*, G_2 phase of the cell cycle; $\bullet$, polysomal poly(A) RNA; $\circ$, nuclear poly(A) RNA.

base sequences than that from S phase (20%) (Fig. 2). The hybridisation reaction of polysomal RNA prepared from S phase or G_2 phase was very similar (Fig. 2).

Differences in RNA synthesis in S phase and G_2 phase of *Physarum* have previously been claimed (for review see ref. 16) and we have since obtained some evidence for replication-transcription coupling¹⁷. The data presented in Fig. 2 suggest to us that replicating DNA may provide RNA polymerase B with other initiation sites for transcription than interphase chromatin, resulting in variable repetitive sequence elements in the nuclear RNA, possibly at the 5'-end of the original transcript. If one could generalise from this finding, we would predict that proliferating cells contain a lower degree of redundancy in their nuclear poly(A)-rich RNA than do resting cells.

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Deletion of α -globin genes in haemoglobin-H disease demonstrates multiple α -globin structural loci

FOUR clinical syndromes are associated with α thalassaemia: (1) the silent carrier state (α thalassaemia-2), with no clinical effect; (2) heterozygous α thalassaemia (α thalassaemia-1) in which a more severe defect in α -chain synthesis produces microcytic red cells, but little or no anaemia; (3) haemoglobin-H disease, usually the combination of α thalassaemia-1 and α thalassaemia-2, resulting in mild to moderate haemolytic anaemia, and (4) hydrops fetalis associated with haemoglobin Barts, an invariably fatal condition which represents the homozygous state of α thalassaemia-1 (for recent reviews see refs 1 and 2). To explain these syndromes, one hypothesis proposes that α thalassaemia-1 and α thalassaemia-2 are caused by a defect of greater and lesser severity affecting alleles of a single α -globin locus³. A second hypothesis is based on evidence that the α -globin structural gene is duplicated⁴; the four syndromes may be the result of involvement of one, two, three or all four α structural genes by thalassaemia. In this study, we tested these hypotheses by measuring the number of α -globin structural genes in haemoglobin-H disease and the results firmly support the latter hypothesis.

Ottolenghi *et al.* and Taylor *et al.* showed that the defect in hydrops fetalis results from complete or partial deletions affecting all α -globin structural genes^{5,6}. This implies that in α thalassaemia-1, half the normal number of α -globin genes are similarly affected. Since some normal α -globin chain is synthesised in haemoglobin-H disease, an intact α -structural gene must be present in the α thalassaemia-2 lesion. If there is one locus for α -chain synthesis, patients with haemoglobin-H disease will also have half the normal number of α -structural genes, but if haemoglobin-H disease is the result of deletion affecting three of the four genes, patients will have a quarter the normal number.

α -Globin genes were measured in a Chinese patient with haemoglobin-H disease with complementary DNA (cDNA) enriched in α -globin sequences. The specificities of this probe and the cDNA enriched in β -globin sequences are shown in Fig. 1. The α and β cDNAs annealed at the same rate and to the same extent to the haemoglobin-A mRNA, but the α cDNA annealed at about one-tenth the rate of the β probe to haemoglobin-H mRNA which contained α : β sequences at a ratio of 1:9. Less than a quarter of the α cDNA annealed to RNA from hydrops reticulocytes, whereas the β cDNA annealed completely. Thus, as much as 25% of the α cDNA used in these experiments could represent contamination with β -globin sequences.

The contamination and the possible partial annealing of the α cDNA with the gene for ζ -globin chain⁵ may com-

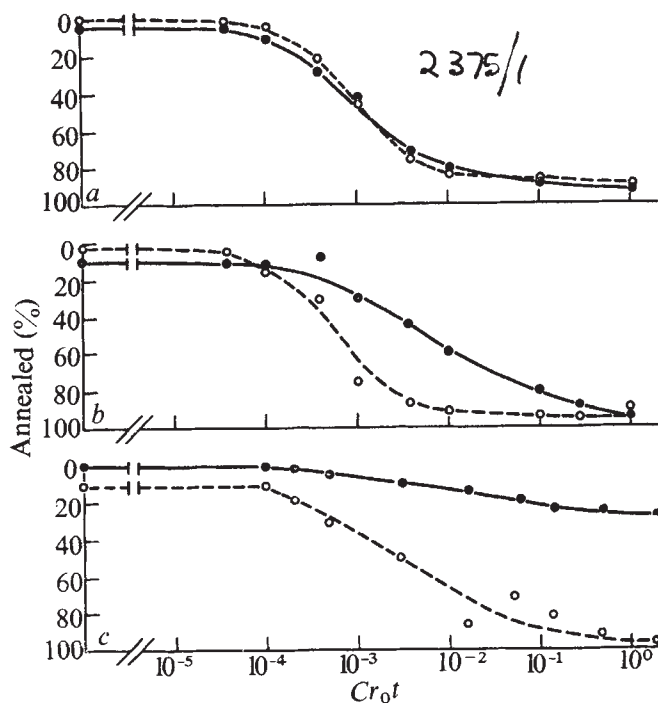


Fig. 1 Annealing of human globin cDNA to globin mRNA. The α cDNA was prepared as described previously⁶. Globin mRNA was isolated from the postribosomal supernatant lysate⁷ of reticulocyte-rich blood of a patient with autoimmune haemolytic anaemia. (This preparation of mRNA directed the synthesis of three times more α chain than β chain in the Krebs ascites cell-free system⁸.) The mRNA was transcribed into cDNA in a 75- μ l reaction mixture which contained 300 U ml⁻¹ avian myeloblastosis virus polymerase, 20 μ g ml⁻¹ RNA template, 5 μ g ml⁻¹ oligo(dT) (PL Biochemicals), 100 μ g ml⁻¹ actinomycin (Calbiochem), 50 mM Tris (pH 8.3), 6 mM MgCl₂, 10 mM dithiothreitol (Calbiochem), 0.5 mM each of TTP, dCTP, dATP and 0.1 mM ³²P-dGTP (New England Nuclear Corp., specific activity 110 Ci mmol⁻¹); and incubated at 37 °C for 30 min. The cDNA was isolated and incubated in the presence of excess template RNA. That cDNA which annealed was isolated on hydroxylapatite and recovered as described⁶. It was then annealed to haemoglobin-H mRNA (α : β ratio = 1:9 on translation in Krebs ascites cell-free system), until 88% of the cDNA was annealed. The single stranded material was eluted from hydroxylapatite and used as the probe for α sequences. The β cDNA was prepared by transcription from the haemoglobin-H mRNA, with ³H- α -GTP (Schwartz-Mann, specific activity 10 mCi mmol⁻¹) as the radioactive label, and was used as a probe for β sequences without further enrichment. The purity of the α and β cDNA was tested by annealing to (a) Hb-A mRNA, (α : β = 1:1), (b) Hb-H mRNA and (c) to hydrops reticulocyte RNA. The conditions of annealing were similar to those described⁶ except that 200 c.p.m. each of ³²P- α probe and ³H- β probe were present together in each reacting mixture. ●, α cDNA; and ○, β cDNA. For (c) it was assumed that 1% of the total RNA was mRNA.

plicate the measurement of α -globin genes. We attempted to circumvent these problems by measuring the rate of annealing of the cDNA to reconstituted mixtures of normal and hydrops cellular DNA. The rate of annealing of the α cDNA to a mixture containing equal parts of each should be similar to that found if half the normal number of α -globin genes are present. The rate of annealing to a mixture containing 1 part of normal to 3 parts of hydrops DNA would represent a quarter of the normal number of α -globin genes. These rates were compared with the rate of annealing of the α cDNA to cellular DNA from a patient with haemoglobin-H disease (Fig. 2). The α cDNA annealed to the haemoglobin-H DNA at a rate identical to the mixture which contained 25% non-thalassaemic cDNA and 75% hydrops DNA. In contrast, the cDNA specific for β -globin genes annealed to all the cellular DNAs and their mixtures at about the same rate.

We conclude that a patient with haemoglobin-H disease has a quarter the normal number of intact α -globin structural genes. This provides direct evidence that a non-thalassaemic person must have at least four α -globin genes per diploid cell. These data do not rule out the possibility that some multiple of four α -globin genes per cell is present. The fact that α -structural abnormalities constitute at least 25% of the total haemoglobin in the heterozygous state⁹, however, makes the higher numbers unlikely.

On the basis of studies of α -globin structural mutants, it has been suggested that the number of α -globin structural genes varies in different populations. The finding of two α -chain mutants, haemoglobins Buda and Pest each comprising about 25%, together with 50% normal haemoglobin-A in the same person in a Hungarian family, firmly supports the two-locus theory¹⁰. On the other hand, the fact that haemoglobin-A is absent in Melanesian homozygous for haemoglobin J-Tongariki^{11,12} and in some Oriental patients with the combination of haemoglobin Q and α thalassaemia-1 (ref. 13), suggests that only one locus is present. The method described here can be used to quantitate α -globin structural genes in these different syndromes.

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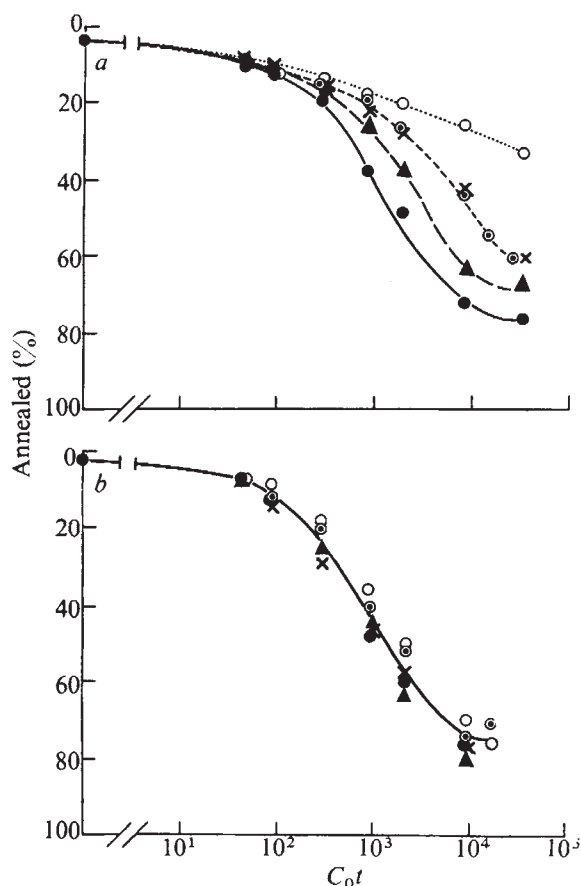


Fig. 2 Annealing of human globin cDNA to cellular DNA. DNA was isolated from the livers of an infant with hydrops and a non-thalassaemic infant, and from the spleen of a Chinese patient with haemoglobin-H disease by methods previously described⁶. The latter patient has a microcytic anaemia with haemoglobin of 10 g, reticulocyte count 4.6%, Hb A₂ 0.9%, Hb F 0.5%, Hb H 15% and no Hb Constant Spring. One sibling studied has heterozygous α -thalassaemia. 200 c.p.m. each of ³²P- α cDNA (a) and ³²P- β cDNA (b) were annealed to cellular DNAs in the following conditions: 5.8 mg ml⁻¹ cell DNA, 0.5 M NaCl, 2 mM EDTA, 40 mM Tris (pH 7.4) at 68 °C, total volume 260 μ l, for 10 min to 120 h. Annealing was assayed with hydroxylapatite. ●, Non-thalassaemic DNA; ○, hydrops DNA; ▲, mixture of equal parts of non-thalassaemic and hydrops DNA; ⊙, mixture of 1 part of non-thalassaemic to 3 parts of hydrops DNA; ×, haemoglobin-H DNA.

Stereochemical basis of heat stability in bacterial ferredoxins and in haemoglobin A₂

Most enzymes are quickly inactivated above about 55 °C, but those from thermophile bacteria are stable for long periods at higher temperatures¹. We do not know why, because so far their structures have proved too complex. For example, although the tertiary and quaternary structures of the enzyme glyceraldehyde phosphate dehydrogenase from lobster muscle and from *Bacterium stearothermophilus* are alike, their amino acid sequences differ by more than 130 out of some 330 positions, which makes it hard to decide why the stearothermophilus enzyme is more stable. The electron transfer protein ferredoxin offers a better chance, because its single polypeptide chain contains fewer than 60 residues; its structure is known, and its heat stability and amino acid sequence have been determined in both mesophile and thermophile bacteria. We have built an atomic model of this protein, replaced its amino acid side chains in turn to correspond to the published sequences and searched for possible causes of the greater heat stability of ferredoxins from thermophile bacteria. We found that it arises mainly from external salt bridges linking residues near the amino terminus to others near the carboxy terminus. Haemoglobin A₂, a minor fraction of adult human haemoglobin which is a little more heat stable than the major fraction, haemoglobin A, seemed another good choice because its amino acid sequence differs from that of A at only 10 positions. The atomic model suggests that at only two of these positions are the replacements likely to contribute to the extra stability of haemoglobin A₂, one replacement providing an extra hydrogen bond between the α_1 and β_1 subunits and the other adding two non-polar interactions to a surface crevice within the β subunits. To account for the increased heat stability of the two proteins, the extra bond energy

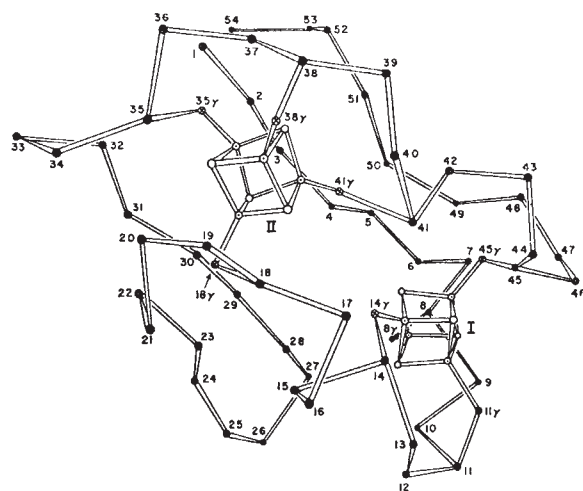


Fig. 1 Plot of α -carbon, iron and sulphur positions in ferredoxin from *M. aerogenes*². (Reproduced by permission of *J. biol. Chem.*)
Fe●; S_{inorg}○; S_{cys}×; C _{α} ●.

provided by these interactions need not be larger than 10 kJ for ferredoxin or 5 kJ for haemoglobin A₂.

The three-dimensional structure of ferredoxin from the mesophile *Micrococcus aerogenes* has been determined² (Fig. 1). It has two active complexes spaced 12 Å apart, each consisting of four atoms of iron and four of inorganic sulphur joined in a distorted cube. The iron atoms of one complex are linked to Cys 8, 11, 14 and 45 and those of the other to Cys 18, 35, 38 and 41. The molecule contains tyrosines in positions 2 and 28, each having one face in contact with one of the Fe₄S₄ complexes and one edge with water.

The thermal stabilities of ferredoxins from two mesophile clostridia (*Clostridium pasteurianum* and *C. acid urici*) and from two thermophile species (*C. tartarivorum* and *C. thermosaccharolyticum*) have been reported³. The amino acid sequences of these four ferredoxins are closely related to the sequence of *M. aerogenes* (Table 1). For instance, the sequences of *M. aerogenes* and *C. thermosaccharolyticum* show thirty identities, including the pattern of the eight

cysteines which are linked to the eight iron atoms. There are ten conservative replacements (for example Ala→Ser; Ser→Thr; Thr→Val; Val→Ile; Gln→Glu) and in eight of these the side chains are external; the two internal replacements are Tyr 2→His at one of the active sites, and Ile→32 Val; both these can be accommodated without change of tertiary structure. There are 12 non-conservative replacements in 11 of which the side chains are external; the one internal replacement Pro 52→Val can be accommodated without change of tertiary structure. Compared to the sequence of *M. aerogenes* the sequences of the four *Clostridium* ferredoxins contain two insertions, one between positions 21 and 22 and the other between 26 and 27, and one deletion at the C-terminus. Fig. 1 shows that the insertions occur at the corners of an external loop and that the C-terminus is also external, so that these modifications can also be accommodated without changing the tertiary structure which seems to be determined primarily by the pattern of cysteines linked to the Fe₄S₄ complexes.

The enzyme from *C. pasteurianum* is completely inactivated after 2 h at 70 °C, that from *C. acid urici* retains 20% of its activity, that from *C. tartarivorum* half, and that from *C. thermosaccharolyticum* 90% (ref 3). To explain these differences we have assumed that the polypeptide chains of all four clostridia maintain a tertiary structure closely similar to that of *M. aerogenes*; we have built an atomic model of the latter and have altered the pattern of side chains in turn to correspond to the four different sequences of *Clostridium*. We have found some of the amino acid replacements such as Thr 5→Asn or Thr 49→Val to be clearly neutral, although others seem highly significant.

For instance, the nature of the aromatic residues in positions 2 and 30, next to the two Fe₄S₄ complexes, varies in different species (Table 2). Ferredoxin from *C. pasteurianum*, which is the least heat stable, has Tyr at 2 and Phe at 30. In *C. acid urici*, which is more stable, Phe 30 is replaced by Tyr; its OH can form a hydrogen bond with the OH of Ser 10. In *C. tartarivorum* and *C. thermosaccharolyticum* Tyr continues to occupy position 30, but Tyr 2, which seems to make no hydrogen bond, is replaced by His which can make a hydrogen bond to the sulphur lone pair electrons of Cys 43. Tanaka *et al.* drew attention to some of the most interesting characteristics of the thermophile enzymes

Table 1 Amino acid sequences of four mesophile and two thermophile ferredoxins

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
<i>M. aerogenes</i> ⁹	A	Y	V	I	N	D	S	C	I	A	C	G	A	C	K	P	E	C	P	V	N	-	I	Q	Q	G	-	S	I
<i>C. pasteurianum</i> ⁷	A	Y	K	I	A	D	S	C	V	S	C	G	A	C	A	S	E	C	P	V	N	A	I	S	Q	G	D	S	I
<i>C. acid urici</i> ⁸	A	Y	V	I	N	E	A	C	I	S	C	G	A	C	D	P	E	C	P	V	D	A	I	S	Q	G	D	S	R
<i>P. elsdenii</i> ¹³	M	H	V	I	S	D	E	C	V	K	C	G	A	C	A	S	T	C	P	T	G	A	I	E	E	G	E	T	K
<i>C. tartarivorum</i> ¹⁰	A	H	I	I	T	D	E	C	I	S	C	G	A	C	A	A	E	C	P	V	E	A	I	H	E	G	T	G	K
<i>C. thermosaccharolyticum</i> ¹¹	A	H	I	I	T	D	E	C	I	S	C	G	A	C	A	A	E	C	P	V	E	A	I	H	E	G	T	G	K
	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56		
	Y	A	I	D	A	D	S	C	I	D	C	G	S	C	A	S	V	C	P	V	G	A	P	N	P	E	D		
	F	V	I	D	A	D	T	C	I	D	C	G	N	C	A	N	V	C	P	V	G	A	P	V	Q	E	-		
	Y	V	I	D	A	D	T	C	I	D	C	G	A	C	A	G	V	C	P	V	D	A	P	V	Q	A	-		
	Y	V	V	T	-	D	S	C	I	D	C	G	A	C	E	A	V	C	P	T	G	A	I	S	A	E	-		
	Y	Q	V	D	A	D	T	C	I	D	C	G	A	C	Q	A	V	C	P	T	G	A	V	K	A	E	-		
	Y	E	V	D	A	D	T	C	I	D	C	G	A	C	E	A	V	C	P	T	G	A	V	K	A	E	-		

Homologies and conservative replacements are boxed. The one-letter amino acid code is as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; Y, Tyr.

Table 2 Aromatic residues in positions 2 and 30 of ferredoxins

Residue no.	<i>M. aerogenes</i>	Thermolabile <i>C. pasteurianum</i>	<i>C. acidu urici</i>	<i>P. elsdenii</i>	<i>C. tartarivorum</i>	Thermostable <i>C. thermosaccharolyticum</i>
Hydrogen bonds gained						
2	Tyr	Tyr	Tyr	His	His	His
43	Cys	Cys	Cys	Cys	Cys	Cys
10	Ala	Ser	Ser	Lys	Ser	Ser
30	Tyr	Phe	Tyr	Tyr	Tyr	Tyr
Invariant salt bridge						
1	Ala	Ala	Ala	Met	Ala	Ala
39	Asp	Asp	Asp	Asp	Asp	Asp
Salt bridges gained						
2	Tyr	Tyr	Tyr	His	His	His
6	Asp	Asp	Glu	Asp	Asp	Asp
7	Ser	Ser	Ala	Glu	Glu	Glu
24	Gln	Ser	Ser	Glu	His	His
25	Gln	Gln	Gln	Glu	Glu	Glu
29	Ile	Ile	Arg	Lys	Lys	Lys
31	Ala	Val	Val	Val	Gln	Glu
44	Ala	Ala	Ala	Gln	Glu	Glu
53	Asn	Val	Val	Ser	Lys	Lys

The salt bridge from Asp 6 to Lys 29 may not be sterically favourable and is therefore shown as a broken line. The numbering of the residues is as in Table 1; to relate it to Fig. 1, subtract 1 from numbers 23–26 and 2 from all numbers above 27. Ferredoxin from *P. elsdenii* is reported to be heat labile¹³ but no quantitative data for its heat stability are available. On the basis of its salt bridges as shown here it should be intermediate between *C. acidu urici* and *C. tartarivorum*.

when they pointed out that glutamine or glutamic acid in positions 31 and 44 can be hydrogen bonded to other amino acid side chains. In fact, glutamates 7 and 25, glutamate or aspartate 6, and aspartate 39 can also form such bonds. Aspartate 39 forms a salt bridge with the α amino group which seems to be invariant. Two of the salt bridges specific to the thermophile enzymes (24–25 and 29–31) are between neighbours or next nearest neighbours in straight segments of the chain and would not necessarily have to break when the chain unfolds, but two others are placed in the most effective positions possible by clamping residues near the amino terminus to residues near the carboxyl terminus (His 2–Gln or Glu 44 and Glu 7–Lys 53). The sequence of the less stable ferredoxin from *C. tartarivorum* differs from that of the more stable one from *C. thermosaccharolyticum* by replacements in only two positions. Glutamines 31 and 44 are replaced by glutamates. His 2 and Lys 29, which are common to the ferredoxins of both clostridia, can form hydrogen bonds with either the uncharged carboxyls of the glutamines or the charged carboxyls of the glutamates; the latter, being salt bridges, are stronger and must therefore account for the extra stability of the ferredoxin from *C. thermosaccharolyticum*. If these conclusions are correct, then the differences between the heat stabilities of the different ferredoxins should diminish on weakening the salt bridges, for example with increasing pH which would discharge histidine 2, or with increasing ionic strength.

In addition to these polar interactions one might have expected the thermophile enzymes to show extra non-polar

interactions, but comparison of the sequences does not point to any position where the thermophile enzymes contain a residue providing consistently more non-polar contacts than any of the amino acid side chains occupying the same position in the mesophile enzymes.

We now turn to human haemoglobin, a protein consisting of four subunits joined in a tetrahedral array. At 45° oxyhaemoglobin A₂ is denatured six times more slowly than A (ref 4). The two proteins differ in only ten positions in the β chains (known as δ in haemoglobin A₂) (Table 3). Seven of these are external, two lie in surface crevices and one lies in the $\alpha_1\beta_1$ contact. Analysis of the external replacements shows that their effect, if any, should be to stabilise haemoglobin A rather than A₂. One replacement (126) in a surface crevice provides an additional non-polar contact between helices A and H within the same β chain of haemoglobin A₂. The replacement in the $\alpha_1\beta_1$ contact (116), introduces one extra hydrogen bond between the guanidinium group of Arg G18(116) β_1 and the carbonyl oxygen of Pro GH2(114) α_1 of haemoglobin A₂ (for illustration of the $\alpha_1\beta_1$ contact see refs 5 and 6.) Dissociation at the $\alpha_1\beta_1$ contact seems to determine the degree of resistance to alkaline denaturation of haemoglobin⁶. The amino acid differences between haemoglobins A and A₂ suggest that the extra hydrogen bond between residues 114 α_1 and 116 δ_2 is decisive in making A₂ more resistant to heat denaturation. If this is correct, then haemoglobin Lepore Boston, a δ - β hybrid in which the N-terminal six residues are δ and the rest β , should have a susceptibility to heat denaturation

Table 3 Interactions in human haemoglobin A and A₂

Residue no.	A β	A ₂ δ	Position	Structural role
Probable gains in A ₂				
116	G18	His	$\alpha_1\beta_1$ contact	Arg forms H-bond to CO of Pro GH2 α
126	H4	Val	Surface crevice	Additional non-polar contact with Val A11(14) β
Probable losses in A ₂				
12	A9	Thr	Surface crevice	Thr OH may bond to CO of Lys A5 β
22	B4	Glu	External	Glu may form weak salt bridge to His G19 β
50	D1	Thr	External	Possible loss of non-polar contact with CH ₃ of Ala D4
117	G19	His	External	See 22
Probable neutral replacements				
9	A6	Ser	External	None
86	F2	Ala	External	Probably none
87	F3	Thr	External	None
124 or 125	H2 or H3	Pro	External	None

similar to that of haemoglobin A, and deoxyhaemoglobin, in which the subunits are joined by salt bridges, should be more heat-resistant than oxy, CO or cyanomethaemoglobin.

In conclusion, thermophile ferredoxins are stabilised by extra salt bridges between external polar groups and by additional hydrogen bonds but not, apparently, by more non-polar contacts. The heat stability of haemoglobin A₂ compared to A is accounted for by one extra hydrogen bond at the contact between the α_1 and δ_1 subunits and possibly two non-polar interactions within the δ subunits.

The denaturation data of ferredoxin refer to irreversible loss of activity accompanied by loss of the iron sulphur clusters from the protein; this requires at least two kinetic steps, unfolding of the chain and dissociation of the cluster, which cannot be separated. Even so the order of magnitude of the extra stabilisation energy provided by the salt bridges and hydrogen bonds can be estimated from the difference in the observed rates of denaturation. The data of Devanathan *et al.*³ suggest that the half times for denaturation ($t_{1/2}$) in the four proteins differ approximately in the order of 1:5:10:20. The corresponding differences in free energy of activation, $\Delta G = RT2.3 \log \Delta t_{1/2}$ amount to 4.6, 6.6 and 8.5 kJ mol⁻¹ at 70 °C for each protein relative to the slowest, assuming G (ground state) to be the same for each protein. This suggests that the total extra free energy of stabilisation provided by the additional bonds in the thermophile ferredoxins is rather less than 10 kJ mol⁻¹. The rate of denaturation at 45 °C of haemoglobin A exceeds that of A₂ about sixfold, which corresponds to an extra stabilisation energy of 5 kJ mol⁻¹. These energies are surprisingly small. Similar conclusions were reached for the difference between the free energy of activation needed to denature triose phosphate isomerase from rabbit muscle and *B.stearothermophilus* (R. C. Fahey and J. I. Harris, unpublished). It seems that in monomeric enzymes the extra energy of stabilisation can be provided without disturbance of the tertiary structure by a few extra salt bridges on the molecular surface; in oligomeric enzymes by some extra salt bridges, hydrogen bonds or non-polar bonds at the subunit interfaces.

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of skeletal and cardiac muscle^{1,2}. Understanding of the regulation of these changes will require analysis of the steps between the initiation of transcription and the degradation of the protein to amino acids. The role of post-translational steps in these processes has generally been ignored since there has been no way to isolate biochemically intermediates of myofibrillar assembly. This is unfortunate since the physiological and metabolic regulation of such steps may control the rate of incorporation of new protein into this organelle, and thus the levels of functional contractile protein in the cell. Present understanding of myofibrillar assembly has come mainly from ultrastructural studies. In embryonic³, regenerating⁴ and rapidly growing muscles from young animals⁵, electron microscopy suggests that polymerised thick and thin filaments can aggregate to form myofibrils *de novo* and/or insert into pre-existing myofibrils. In contrast, microscopy of normal adult muscle usually does not reveal these structural aspects of myofibrillogenesis, although isotope studies showed that contractile proteins are constantly synthesised and degraded in the muscle cell^{1,2,6}, even in the absence of net growth. This may be because only a small fraction of protein exists as nascent filaments not yet integrated into fibrillar sarcomeres, and/or because these assembly steps may be too rapid to be captured by electron microscopy. We report here the isolation of a population of thick and thin myofilaments that may constitute such an intermediate in myofibrillar assembly.

Female Sprague-Dawley rats (200–220 g) were killed and the muscles excised, minced and soaked in an EGTA-pyrophosphate containing relaxing solution, and then homogenised in a solution containing EGTA. The homogenate was centrifuged at 800g to sediment myofibrils and centrifugation was repeated until the supernatant was devoid of myofibrils. Electron microscopy of this fraction revealed the presence of numerous free thick and thin filaments which tended to form 'bundles' through lateral and longitudinal aggregation. By supplementing the post-myofibrillar supernatant with 2 mM ATP, the aggregation of these myofilaments could be prevented, in turn making clearer visualisation of these filaments possible by negative staining and the subsequent centrifugation of this fraction at 10,000g for 10 min to remove additional subcellular material from this fraction. Finally, these filaments were purified further by treatment with 1% Triton X-100 based on previous reports that no myofibrillar components are extracted with this non-ionic detergent⁷. Figure 1 illustrates a field of such filaments after this purification. The amount of protein in this preparation, measured by the Lowry method⁸, varied with the degree of homogenisation of the muscle but was between 10% and 15% of total myofibrillar protein when homogenisation was carried out for 30 s at 14,000 r.p.m. in an MSE blade type homogeniser.

Since the release of filaments in the whole muscle homogenate is likely to depend in part on endogenous muscle ATP necessary for actin-myosin relaxation⁹, we quantified the amount of myofibrillar protein found in the purified filaments as a function of the time that minced muscle was soaked before homogenisation. When this step lasted 3 h, with repeated replacement of the washing solution, virtually no protein was detected in this preparation and no free filaments or aggregated filaments were found in the crude muscle homogenate examined with the electron microscope. Thus, the free and/or loosely held filaments are probably trapped on the surface of the myofibrils through, in part, rigour-links caused by washing out of endogenous ATP from the mince. This suggested an alternative method for isolating fresh homogenate filaments, based on their entrapment on myofibrils and subsequent release. Myofibrils were, therefore, prepared from this 'rigour homogenate' and gently treated with Mg-ATP relaxing solution, resulting in the release of approximately 10–15% of myofibrillar protein in the form of thick and thin filaments. When myofibrils were

Isolation of newly synthesised myosin filaments from skeletal muscle homogenates and myofibrils

LARGE and rapid changes in the concentrations of myofibrillar protein can occur during hypertrophy and atrophy

viewed with the phase contrast or electron microscope during treatment with Mg-ATP relaxing solution, they seemed at first to stiffen, followed by a swelling and fanning out of filaments at the periphery of the fibrils, suggesting the release of filaments from the fibrillar periphery. In related studies, we have shown that the whole homogenate filaments and filaments released from fibrils lack M-line protein(s) of 190,000 and 170,000 daltons and Z-line protein(s) of 90,000 daltons, and have decreased amounts of protein larger than 200,000 daltons (ref. 7 and J. D. E., unpublished).

To investigate the significance of the easily releasable filaments, the rats were first injected with tritiated leucine into the tail vein after a 24 h fast. After times ranging from 5 min to 24 h, the animals were killed and the isolated filaments were prepared from the whole homogenate directly or through entrapment on the myofibrils and subsequent filament release. The residual undispersed myofibrils were also prepared in each case. The preparations were electrophoresed on 5% SDS-acrylamide gels and the stained myosin heavy chain band was sliced and hydrolysed (Fig. 2). Tritium was counted in one portion of the hydrolysate and the remainder was assayed for leucine (Table 1).

The specific activity of the myosin heavy chain in the filament fractions was substantially and reproducibly higher than that obtained from residual myofibrils (Table 1). Furthermore, the differences in these specific activities

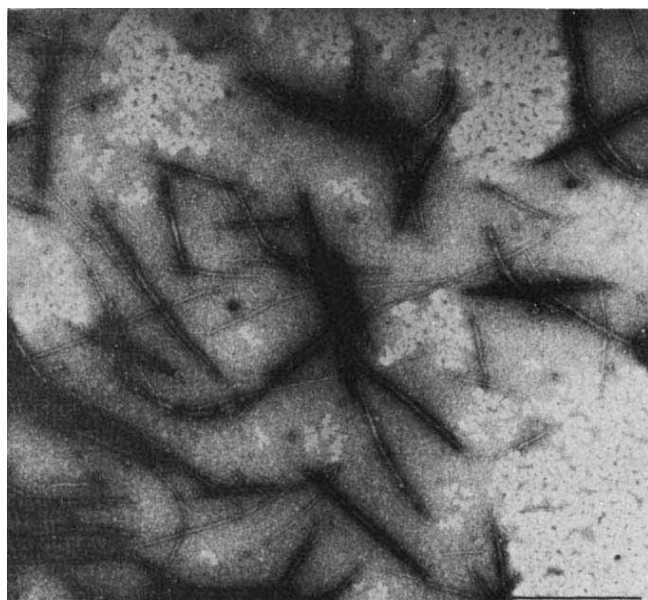


Fig. 1 Electron micrograph of thick and thin filaments isolated from the post-myofibrillar supernatant fraction of a fresh muscle homogenate. Mixed rat back muscle was excised and coarsely minced in 0.1 M KCl, 2 mM MgCl₂, 2 mM EGTA, 2 mM Na₂P₂O₇, 1 mM dithiothreitol (DTT), 0.01 M Tris-maleate, pH 6.8 at 0 °C. The muscle was then disrupted with an MSE homogeniser for 30 s at 14,000 r.p.m. in 10 volumes of a solution of the same composition without pyrophosphate, followed by 5–10 strokes in a glass Dounce homogeniser. The homogenate was filtered through two layers of cheesecloth and centrifuged at 800g for 10 min. This supernatant was centrifuged again at 800g for 10 min and then adjusted to a final ATP concentration of 2 mM to prevent aggregation of myofilaments. The ATP-supplemented filament preparation was then centrifuged at 10,000g for 10 min. The post-10,000g supernatant was then supplemented with Triton X-100 to yield a final concentration of 1%, and placed on ice for 5 min. Next CaCl₂ was added to a final concentration of 2 mM and, after 15 min, the aggregated filaments were pelleted by centrifugation at 15,000g for 1 h. These filaments were then redispersed in 0.1 M KCl, 2 mM MgCl₂, 2 mM EGTA, 2 mM ATP, 1 mM DTT, 0.01 M Tris-maleate, pH 6.8 (Mg-ATP relaxing solution) and negatively stained with aqueous 2% uranyl acetate. The calibration bar equals 0.5 μ m.

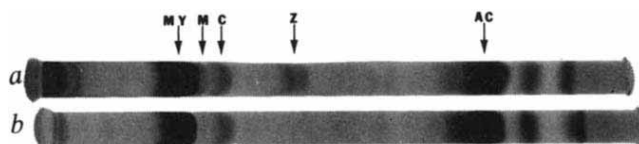


Fig. 2 SDS gel electrophoresis of filaments and residual myofibrils isolated from a fresh muscle homogenate. Isolated filaments were prepared as described in Fig. 1 and the 800g pellet obtained from the muscle homogenate was used to obtain myofibrils. The myofibrils were prepared by washing the pellet six times with 0.1 M KCl, 2 mM MgCl₂, 2 mM EGTA, 1 mM DTT, 0.01 M Tris-maleate, pH 6.8, once with the same solution containing 1% Triton X-100, and twice without Triton. Each preparation was then electrophoresed on 5% SDS-acrylamide gels as described previously¹². Similar patterns are obtained when filaments are released from fibrils although the proportions of myosin and actin varied between preparations. A, residual myofibrils; B, thick and thin filaments; MY, myosin heavy chain; M, M-line protein(s); Z, Z-line protein(s); C, C-protein; AC, actin. Assignment of protein bands is according to ref. 7.

seemed to decrease with time after isotope injection. The precise time dependence of this difference must, however, be regarded as tentative because small variations in filament yield may affect the exact difference obtained in a given experiment. We have also obtained similar results in the heart (J. Sims and R. Z., unpublished). The quantitation of this phenomenon and its applicability to other myosin and myofibrillar components is being studied.

In most protocols for the isolation of contractile proteins, the 'insoluble' myofibrillar fraction serves as the starting point for extraction and purification. Discarding the supernatant fraction which we have found to contain myosin filaments of substantially higher specific activity could seriously affect estimates of protein synthetic rates and protein half lives based on these rates. In the whole animal system used here, apparent rates of synthesis would seem lower than the actual rates, particularly at the earlier time points¹⁰.

The myofilaments found in the supernatant fraction must be either filaments which are not as tightly incorporated into

Table 1 Standard specific radioactivity of the myosin heavy chain in filament fractions and in residual myofibrils

Experiment	Time after injection	Filaments (standard specific activity)	Residual myofibrils (standard specific activity)
1	5 min	2,648	767
2	5 min	3,620	695
3	5 min	1,453	586
4	30 min	2,263	852
5	3 h	2,873	1,513
6	24 h	2,885	2,027

Rats were injected with 300–2,000 μ Ci of ³H-leucine. In experiments 1 and 2, filaments and myofibrils were isolated directly from the fresh whole muscle homogenate as described in Fig. 1, Fig. 2 and in the text. In experiments 3 to 6, filaments were released from 'rigour homogenate' myofibrils. Filaments were released from the myofibrils by 10 passages through a Pasteur pipette of the fibrils in Mg-ATP relaxing solution (Fig. 1). The filaments were separated from undispersed fibrils by centrifugation at 800g for 10 min after which the supernatant was recentrifuged at 800g for 10 min. The filaments in the 800g supernatant were pelleted by centrifugation at 100,000g for 3 h. The myosin heavy chain band (MY) was sliced from the stained gels (Fig. 2). A 3 mm slice containing 20–50 μ g protein was hydrolysed in 5 ml of 6 N HCl–0.1% mercaptoethanol at 110 °C for 24 h. The samples were then placed at 0 °C for about 12 h and then centrifuged at 500g. The supernatant was evaporated to dryness and the precipitate was washed three times with H₂O by evaporation. Samples were resuspended with 1.3 ml of H₂O and then 1 ml was counted in 10 ml of Scintisol with a Packard liquid scintillation counter. 0.2 ml was used to assay for leucine according to the isotope dilution method of Rubin and Goldstein¹³. Results are expressed as standard specific activity, = d.p.m. μ mol⁻¹ leucine $\times$ rat weight (g) per μ Ci injected.

the fibrils or filaments which actually exist in a cytoplasmic pool before their attachment to fibrils. (Since the amount of myofibrillar protein recovered in the supernatant fraction at least partly depends on the extent of tissue disruption, a portion of these filaments must be released from myofibrils.) Further experiments do not support the hypothesis that the high specific activity filaments arise from the preferential release of filaments from the myofibrils of red compared with white fibres (our work in preparation).

Morkin's electron microscopic autoradiographic studies of postnatal skeletal muscle suggested that newly synthesised myofilaments are added to the periphery of pre-existing fibrils¹¹. It is likely that we have, at least in part, obtained a preferential separation of these newly synthesised peripheral filaments from the less dissociable 'core' myofibrils. In non-growing heart muscle or skeletal muscle undergoing little or no increase in length, peripheral addition would seem to be a reasonable way to add new myofilaments. In hypertrophying heart muscle or in skeletal muscles from prenatal or young animals, which are rapidly lengthening along the fibre axis, addition of new filaments to the ends of myofibrils^{3,14} may also be important.

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A similar law may govern water freezing in minerals and living organisms

THERE is an interesting similarity between the freezing of water in minerals and in biological tissues. Mazur^{1,2} observed that many living organisms lose viability at threshold temperatures near -10°C . After investigating this phenomenon from the point of view of possible mechanisms by which living cells suffer damage on freezing, he concluded that a key step in the freezing process is the penetration of ice into the cell to nucleate the internal water, and that at -10°C ice can penetrate the membrane, whereas above this temperature, it cannot. This implies that the water in the cell-membrane pores or a portion

of them, starts to freeze at about -10°C . From the Kelvin equation which describes freezing in terms of the ice-water surface tension and the radius of cylindrical capillaries, Mazur deduced that¹ "the lower melting point of ice in a capillary can account for the barrier properties of the plasma membrane". He observed, however, that "there is a large quantitative discrepancy. The evidence suggests that the plasma membrane ceases to be a barrier at approximately -10°C . On the basis of equation (4), (ref. 1) the critical pore radius at -10°C is $30\text{ \AA}$, whereas the estimated pore size from permeability studies is $3-8\text{ \AA}$ ". To account for the discrepancy he postulated that the contact angle of water in the membrane pores is not 0° but 80° . This enabled him to find the simple relationship

$$\Delta T \approx 52/a \quad (1)$$

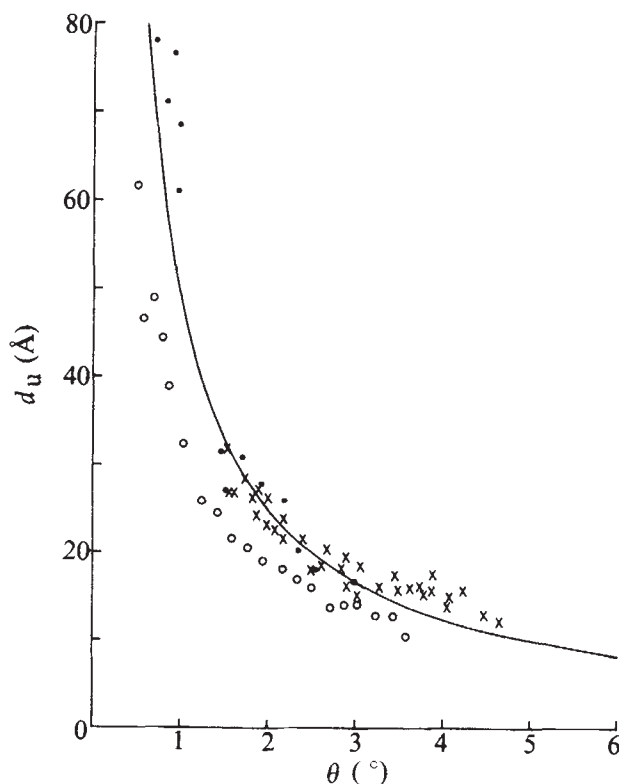
where ΔT is the freezing point depression and a is the pore radius in $\text{\AA}$, which predicts that water in $5\text{ \AA}$ pores will freeze at -10°C and thus agrees with experimental observations on membrane pore sizes.

This result is strikingly similar to an equation that we have found governs the thickness of the unfrozen water layer, d_u , on mineral surfaces:

$$d_u \approx 50 (1/\theta) \text{ or, rearranged, } \theta \approx 50/d_u \quad (2)$$

where d_u is in $\text{\AA}$ and θ is temperature in degrees below 0°C ; d_u is calculated from experimental results and is equal to the ratio of the volume of unfrozen water V , to the specific surface area of the solids S . For non-porous adsorbents or for porous systems in the unsaturated state, that is, when their pores are not completely filled with the adsorbate (a substance capable of being adsorbed but which is not in the adsorbed state) or adsorbate, d_u is considered to be the statistical average thickness of the layer of unfrozen water. In saturated porous systems the ratio V/S is equal to the hydraulic radius, r_h of the pores. For cylindrical capillaries, often used as convenient but probably not

Fig. 1 Measured relationships between the average statistical thickness of the layer of unfrozen water (d_u) and temperature below 0°C (θ) for three different mineral materials. Also included is the smoothed theoretical curve fitted to the results (continuous line, $d_u = 50\theta^{-1}$). $\times$, Kaolinite; $\bullet$, basalt; $\circ$, limonite-goethite.



very realistic models of pores, $r_h = r/2$ where r is the capillary radius. For slit-type capillaries, $r_h = d$ where d is half the distance of separation between the plates; slit-type capillaries are a reasonable model for pores between plate-shaped minerals such as the aluminosilicates. The value of the coefficient in equation (2) was derived from measurements⁹ of the unfrozen water content as a function of temperature on several partially frozen, water-saturated, mineral-water mixtures, each differing quite considerably in their crystallographic structure and physicochemical properties. These data for powdered basalt (a primary, basic rock), limonite-goethite (iron oxide) and kaolinite (an aluminosilicate mineral) are depicted in Fig. 1. The fitted curve is simply a plot of equation (2). We discuss elsewhere how deviations from this freezing law may arise in porous bodies as a result of differences in distribution of pore size, and how these may affect the calculated thickness of unfrozen water layers at temperatures near 0 °C. At lower temperatures, however, (−6 to −10 °C) when the thickness of the unfrozen water layer lies in the range of 5–8 Å, water-ice phase composition data for most mineral materials fall on the same temperature-thickness curve. Note that the similarity between these three different materials would not have been as apparent if instead of layer thickness, unfrozen water content was plotted as a function of θ using the more common units of g H₂O/g solid, because of differences in specific surface areas between the various materials.

We will further analyse the possible physicochemical basis for the freezing process. Our hypothesis may be elaborated as follows: In the force field caused by the forces emanating from the solid or resulting from surface curvature of the liquid in capillary pores, the chemical potential or the vapour pressure of the water is lowered compared with that of bulk pure water. The smaller the capillary and the thinner the layer of adsorbed water, the lower the water chemical potential. When freezing is initiated at 0 °C, only the water that has chemical potential equal to free water solidifies whereas water in narrow pores, or affected by the solid surface and therefore having lower potential, remains unfrozen. As the temperature of the system is lowered further, the chemical potential of water, in the form of ice, is correspondingly decreased⁴ creating a gradient that successively induces increments of the unfrozen water to solidify. Thus, two condensed phases of water can coexist at temperatures below 0 °C—that is, unfrozen liquid water and ice crystals. The unfrozen water can be present either in narrow capillaries or as thin adsorbed layers. At each temperature the chemical potential of the unfrozen water is equal to that of ice at this temperature, being in thermodynamic equilibrium and physical juxtaposition with the ice. The relationship of thickness of unfrozen water to temperature can therefore be viewed as dependent on (and also a measure of) the change in chemical potential of water with the radius of pores or the distance from the solid surface.

The mode of change in chemical potential of a capillary condensate with pore size is well established; the Kelvin equation is a special case applicable to cylindrical capillaries. In general, the chemical potential depends on the nature of the liquid (its molar volume and surface tension) and is independent of the nature of the solid composing the walls of the pores. For cylindrical and slit-type capillaries it is proportional to r_h^{-1} . For thin adsorbed layers it was shown for nitrogen^{6,7} and for water⁸, that adsorption data relating some measure of the chemical potential (vapour pressure) to the thickness of adsorbed layer for many solid adsorbents fall on a common curve termed by de-Boer and his associates⁶, the standard t curve. Thus the t curve seems to be characteristic of the adsorbate or adsorptive but virtually independent of the adsorbent. From these standard t curves it is also found that the chemical potential of the adsorbate usually varies with d^{-3} as expected for the classical van der Waals interaction resulting purely from dispersion forces acting between an atom and a slab⁹. Accordingly, and in view of the previous discussion on the mode of near-surface water freezing, we suggest that many porous bodies and adsorbents may have a common freezing curve relating the

layer thickness of unfrozen water or the hydraulic radius of pores containing them, to temperatures below 0 °C. Our data show that this is the case for a variety of minerals (Fig. 1). From the data of Mazur it seems also to be the case for biological systems.

One puzzling and unresolved finding is that the coefficient in equations (1) and (2) relating the temperature below freezing point to the hydraulic radius or the equilibrium thickness of water on the surface (or when converted to other units, relating the chemical potential to the distance from the surface) is smaller by a factor of five to ten than that predicted from the Kelvin equation. The tentative explanation of Mazur¹ that the contact angle of the water in the pores is not 0 ° but 80 ° does not seem to be sufficient in view of the fact that three different adsorbents are involved in deriving the relationship given in equation (2). Also, the fact that even at every close proximity to the solid the interaction still seems to vary with d^{-1} rather than with d^{-3} , is puzzling. Thus none of the presently known modes of interaction between solid porous adsorbent and liquid adsorbate seems to account completely and quantitatively for these experimentally determined generalised freezing relationships.

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⁹ Adamson, A. W., *Physical Chemistry of Surfaces*, 330 (Interscience, New York, 1967).

Corrigenda

In the article "Evidence that mitogenic lectins induce changes in membrane fluidity" by R. E. Barnett, R. E. Scott, L. T. Furcht and J. H. Kersey (*Nature*, **249**, 465; 1974) the authors request that the following changes be made to the method. "The organisation of the membrane lipids was probed with a mixture of 3 spin labels, methyl 6-(4',4'-dimethyl-oxazolidinyl-N-oxyl)-heptadecanoate (10%), N-(1,1-dimethyl-2-hydroxyethyl)-6-(4',4'-dimethyl-oxazolidinyl-N-oxyl)heptadecamide (30%), and 1-[2-(1,1-dimethyl-2-hydroxyethylimino)cyclopentyl]-1-(4',4'-dimethyl-oxazolidinyl-N-oxyl)undecane (60%). The observed differences in the order parameter could also be caused by differential uptake or metabolism of the labels by the cells."

In the article "Role of f-gravity in cosmological models" by C. Sivaram, K. P. Sinha and E. A. Lord (*Nature*, **249**, 640; 1974) equation (2) should have read

$$\dot{R}^2 = \left(\frac{2}{3}\right)\pi G\rho R^2 + \left(\frac{2}{3}\right)\Lambda c^2 R^2$$

The Λ -term occurs with a *positive* sign, so that a minimum in the function $R(t)$ will occur only if the coupling constant G_f is *negative* (and Λ_f positive). Thus the conclusions of this paper are valid only if the coupling constant of strong gravity is taken to be negative. The authors wish to thank J. V. Narlikar for pointing this anomaly out to them.

reviews

MOLIERE'S hero, Monsieur Jourdain, was amazed to learn that he had been speaking prose for 40 years. Is it really true that he spoke not just prose, but deep structures and transformations? Knowledge is a fine thing, indeed; but the question prompts another more serious one. How could it be shown that transformational grammars are wrong in principle?

One approach would be to establish the existence of certain sentences that they could not properly characterise. But grammatical transformations are an exceedingly powerful computational device, and it is very doubtful whether there are any such sentences. Another approach would be to show that no formal device could specify what sequences of words are grammatical and what sequences are ungrammatical. But even if this thesis were to be proved, which is extremely unlikely, it would establish too much. It would show that language was not governed by any determinate set of rules. Perhaps the general idea of transformational grammar is empirically invulnerable, and it remains only to establish the best grammar within its confines—a view which seems to be held by its current practitioners. There is, however, one aspect of the general theory that may be potentially testable. Transformational grammar should provide the basis for a psychology of language. It is this thesis, initially advanced by Chomsky, that Fodor, Bever and Garrett have set out to explore. Their original disciplines were, respectively, philosophy, linguistics, and psychology; and as one might expect from so talented an interdisciplinary team they have produced a comprehensive and thoughtful book.

It is not difficult to appreciate why transformational grammar has proved so popular with psycholinguists. The authors review the deficiencies of its linguistic predecessors, the taxonomic grammars of the structuralist tradition, and show how neatly these grammars matched the outdated psychological assumptions of neobehaviourism. As Chomsky was one of the first theorists to point out the linguistic deficiencies of taxonomic grammars, it is scarcely surprising that his alternative approach to language was so appealing. His key assumption was that each sentence is derived by a series of transformations from an underlying deep structure specifying its fundamental grammatical relations. Later, of course, he came to

believe that there is an interpretative component that also operates on the deep structure of a sentence in order to yield its essential meaning. So the crucial empirical test is whether it is possible to find traces of such structures and processes in a person's production and understanding of sentences. After one early inspirational article in *Psychology Today* (the American version), Chomsky was quick to close this empirical loophole. He invoked his notorious distinction between a speaker's knowledge or linguistic com-

survey of studies of language acquisition. There is little evidence that children actually acquire grammatical transformations; there is, however, evidence that the order in which they master different sorts of sentence depends upon how difficult it is to relate them to their corresponding deep structures.

What an extraordinary state of affairs. It is as though each act of a play ended with a tableau appropriate, say, to *King Lear* although it had no discernable plot (or else it turned out to be that of *Charley's Aunt*). This is a problem that the playwright can resolve only by such time-honoured devices as, "with one bound the hero was free". Unfortunately, Fodor, Bever and Garrett have been unable to untie their hero; but there is perhaps an alternative explanation of the psychological phenomena. Indeed, looking closely at the text, one discovers such a possibility, which the authors have in their hands but let slip. They write (p. 270): "... so long as the deep structure is assumed to be that syntactic representation of a sentence which is semantically interpreted, hypotheses about the psychological effects of deep structure features tend to be confounded with hypotheses about the psychological effects of semantic relations". This confounding factor is the crux of the matter. One of the authors' typical experiments establishes that when a click is presented in the middle of a sentence, its apparent location migrates towards a boundary between clauses. The migration occurs even if there is no explicit boundary in the surface structure of the sentence. The authors accordingly argue that deep structure clauses have a perceptual integrity that leads to the relocation of events which interrupt them. Yet the crucial factor could be, not the coherence of a clause's underlying syntactic relationships, but the coherence of its meaning. Transformational grammar is, however, by no means the only grammatical theory to assume that clauses have a meaning: proponents of Halliday's systemic grammar, for example, may be equally heartened by the conclusion that the semantic denouement is more important than the syntactic staging.

The main weakness of the book is the result of the authors' reluctance to look over the fence into their neighbour's backyard. They do not consider any of the current alternatives to transforma-

Is all that is not verse just prose?

P. N. Johnson-Laird

The Psychology of Language: An Introduction to Psycholinguistics and Generative Grammar. By J. A. Fodor, T. G. Bever and M. F. Garrett. (McGraw-Hill Series in Psychology.) Pp. xvii+537. (McGraw-Hill: New York and London, 1974.) £8.80.

petence and how he exercises this knowledge in actual linguistic performance. The grammar is intended to apply only to linguistic competence—an assumption that has proved baffling to the point of apoplexy for those psychologists working within the Empiricist tradition.

A theory of linguistic performance might, however, incorporate a theory of competence couched in the form of a transformational grammar. Fodor, Bever and Garrett review the psycholinguistic literature in order to evaluate this proposition. They consider in detail studies of the production, perception and memory of sentences, naturally concentrating on the work that they and their colleagues have carried out at MIT. The moral they draw from their review is simple, but equivocal. There is evidence for the psychological reality of the notion of deep structure; there is little evidence for the psychological reality of processes corresponding to grammatical transformations. The authors draw a similar moral from their

tional grammar and, in particular, the approach to language developed by the artificial intelligentsia working with computers. One suspects that they made a deliberate editorial decision to exclude this work—a great pity because many of the issues that they fail to resolve look more than susceptible to a procedural approach. In most other respects, however, the book is successful. Again and again, one is impressed by the authors' ability to think beyond an 'obvious' approach to a topic. They take a discerning route through the literature; and it is a real intellectual pleasure to follow in their footsteps.

The book is sophisticated but accessible. It is the definitive defence of the relevance of Chomsky's work to psycholinguistics, unlikely to be surpassed in its scope or thoroughness. Yet the defence fails. At the one point at which the general theory of transformational grammar is empirically vulnerable, it is all too vulnerable. The book probably marks the end of an era. It is unlikely that anyone will ever again mount so comprehensive a defence of transformational psycholinguistics. Monsieur Jourdain may go back to speaking simple prose. □

Slipper animalcule

Paramecium: A Current Survey. Edited by W. J. Van Wagtendonk. Pp. xviii+499. (Elsevier Scientific: Amsterdam, London and New York, 1974.) \$53.90.

THE genus *Paramecium* is familiar to most biologists and to many other scientists because it occupies a place in most courses on elementary biology. The amount of attention that has been paid to it in biological research is reflected in the fact that it has been the subject of more than 4,000 scientific papers and 4 monographs, the most recent of which is this volume edited by Professor Van Wagtendonk.

The book contains eight chapters contributed by workers actively engaged in research using *Paramecium*, and is said to provide "an up-to-date evaluation of research on all aspects of *Paramecium* biology." The eight subjects covered are: morphology, taxonomy and general biology; mating types; surface antigens; behaviour and motor responses; growth patterns and morphogenesis; structure; nutrition; and intracellular particles. Most chap-

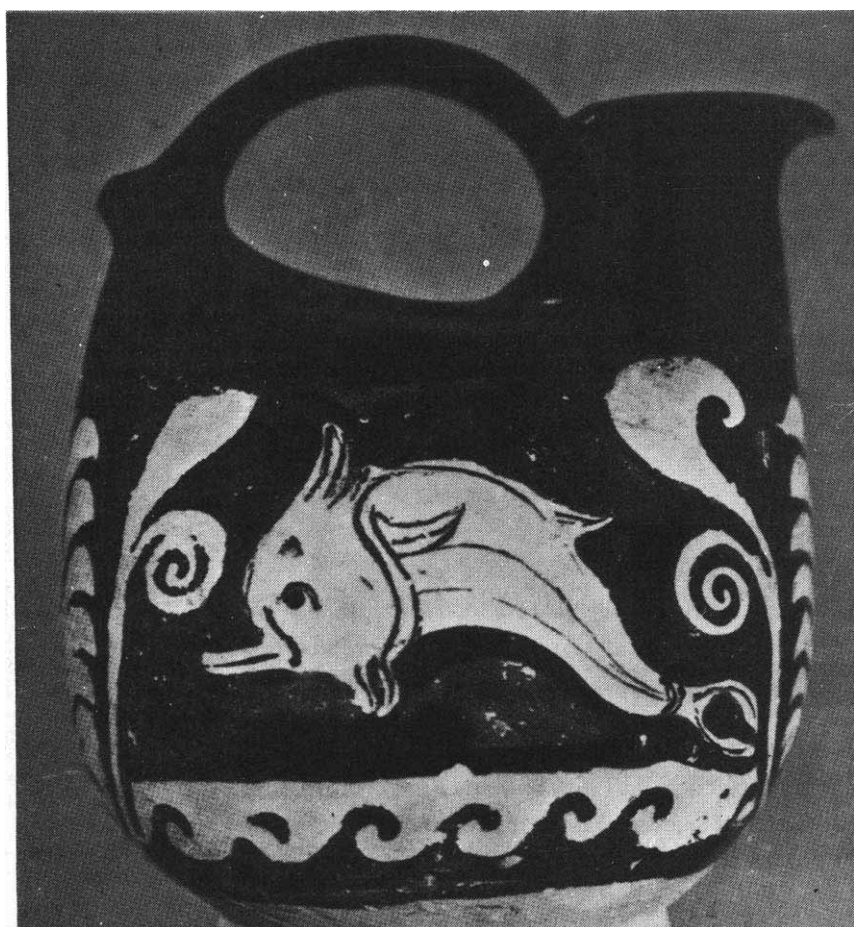
ters refer to the literature published up until 1969 or early 1970, though all are provided with addenda in which reference is made to work of the following two or three years. There is also a comprehensive bibliography of publications on *Paramecium* since 1953, the date of an earlier monograph on the subject by Wichterman.

The general introductory chapter by Vivier will be found the most useful by the average reader because it can be understood without much previous knowledge of the organism. This is not generally true of the other chapters, several of which assume an extensive background knowledge about the organism and specific facets of its biology, to which one is not introduced elsewhere in this book; most readers will not find such chapters fully comprehensible without preliminary reading of earlier work. All of the authors have, however, given an interesting account of the new knowledge being discovered at the frontiers of research upon *Paramecium* and all of the chapters contain much useful information for the protozoologist and cell biologist engaged in research and teaching, which recommends this volume as a valuable work of reference.

The reader will not find here any extensive information on nuclear function, on general physiological activities such as ionic regulation, contractile vacuole function or respiration, or on a number of minor facets of research on *Paramecium*, so that the monograph is not a comprehensive work. In a chapter on nutrition one might expect to find an account of the types of food eaten and the manner of entry into the body; not just a very detailed survey of the chemical dietary requirements for the growth of the animal. The view that mating types correspond to sexual types (p. 68) is less satisfying than the view that every *Paramecium* is hermaphrodite, producing gametic nuclei of both sexes, with mating types acting as devices to encourage cross fertilisation, analogous to self sterility and similar mechanisms that ensure cross pollination in plants. In the magnificently illustrated chapter on structure, a conventional analytical approach would have more successfully presented the information than the attempted synthetic method by which the authors built up a view of the structure from the component molecules, macromolecules and organelles.

The book is illustrated with a large number of electron micrographs and many other figures of a uniformly high standard. Errors and omissions in the figures, legends and tables are few, but are irritating and probably could have been avoided, although there are commendably few spelling errors.

Michael A. Sleight



A Greek dolphin ceramic from the fourth century BC. From *Dolphins (The Undersea Discoveries of Jacques-Yves Cousteau)*. By Jacques-Yves Cousteau and Philippe Diolé. Translated by J. F. Bernard. Pp. 304+105 colour photographs. (Cassell: London, May 22, 1975.) £5.00.

Astrophysical standards

Astrophysical Formulae: A Compendium for the Physicist and Astrophysicist. By Kenneth R. Lang. xxvii+735. (Springer: Berlin and New York, 1974.) \$78.80.

IN view of the enormous range of topics at present covered by astrophysics, it might be thought that a book which "is meant to be a reference source for the fundamental formulae of astrophysics" from nucleosynthesis to astrometry, would automatically be doomed to failure; but Kenneth Lang's attempt succeeds to a remarkable extent. The subject is divided into seven topics, grouped into five chapters—continuum radiation; monochromatic radiation; gas processes; nuclear astrophysics and high energy particles; astrometry and cosmology—and each topic is developed from first principles. This treatment, and the inclusion of several figures and tables of physical data, may make the book useful to the non-astronomical physicist, as promised in the subtitle, although beyond the basic development of each section the bias becomes increasingly too astrophysical for general use.

The disadvantage of this approach is that the discussion of any particular type of astronomical object becomes scattered throughout the text: neutron stars thus occur in such contexts as 'cosmology' (collapse of massive body), 'nuclear astrophysics' (URCA process), 'gas processes' (equation of state) and 'high energy particles' (X-radiation accretion in binary systems); similarly, the table of pulsar properties appears within the chapter on continuum radiation where the formula for dispersion measure is derived. (The pages of this table, incidentally, are printed in the wrong order.)

The comprehensive, 47 page, index minimises this drawback, and also allows quick access to the 69 tables of physical and astronomical data, the latter ranging from the planetary satellites through globular clusters to radio galaxies and quasars. A listing of the tables and figures in the contents section would have been useful though none is given. There are the inevitable misprints, for example "NGC 1270" for 1275 on page 554, and " $\log M_v$ " instead of M_v in the stellar luminosity function (Fig. 43); and there are also some omissions: Saturn X is missing from the list of satellites and the eccentricity and inclination of Pluto's orbit are not given in Table 57.

By its very nature a book of this kind cannot include enough information for day-to-day research, and its price too is likely to make it appear

predominantly on library shelves. There is no doubt, however, that it will be an important reference work for any astrophysicist requiring basic information in a field outside his own, not just because of the concise presentation and clear explanation of the results, but also because it presents nearly 2,000 references, which include original sources from as far back as 125 BC (Hipparchus). This book should for many years remain one of the standard reference works in astrophysics.

Nigel Henbest

Slime organisms

The Mycetozoa. By Lindsay S. Olive. Pp. x+293. (Academic: New York and London, January 1975.) \$23.50; £11.30.

THE slime molds, both cellular and acellular have become increasingly popular over the last few years. Yet I suspect that most experimental biologists have little appreciation of the wealth of forms that exist and of how many riches are waiting to be tapped. In this book, Lindsay Olive does two things. He gives an admirable summary of some of the experimental studies of *Dictyostelium* and *Physarum* and, more important, discusses all the lesser known forms that are related in various interesting ways.

This is a monograph of the Mycetozoa in the classical sense. It discusses the taxonomy, the phylogeny, the morphology (including modern electron microscopic cellular morphology), and, in many cases, the experimental biology of all the known slime molds. What is most extraordinary is that many of the unusual organisms, some of which become 'missing links' between major groups, have all been described by Lindsay Olive himself. One would have thought that all the main types of organisms had been discovered long ago, but in the tradition of Roland Thaxter, Lindsay Olive, often in collaboration with Carmen Stoianovitch, has described many new species and whole new groups of organisms that were quite unknown until a few years ago. Because of the radical nature of these forms, Olive has not followed the well worn position of the past that myxomycetes and cellular slime molds be considered fungi. The main reason for this tradition was that the organisms were discovered by mycologists. Even though Olive himself is a mycologist he has taken the global view that the slime organisms are more closely allied to the protozoa and has fearlessly called them all Mycetozoa. This fine book, therefore, has much to offer to the protozoologist, the mycologist, the cell biologist, and the developmentalist.

J. T. Bonner

Calcium and secretion

Calcium and the Secretory Process. By Ronald P. Rubin. Pp. xiii+189. (Plenum: New York and London, 1974.) \$18.00.

THE central importance of calcium in a number of secretory processes—especially those in which secretion occurs by exocytosis—is now well established and Dr Rubin has provided us with a useful survey of the present position.

His book is divided into an introduction and three main chapters. In the first he reviews in considerable detail evidence for a role of calcium in a variety of secretory systems. The information presented in this chapter is excellent but suffers somewhat from a lack of balance in approach. The classical model system for the study of calcium in neurosecretion, the neuromuscular junction, is discussed only cursorily whereas many pages are devoted to discussions of whether calcium participates in the secretion of, for instance, the thyroid and other glands. In spite of the existence of many good reviews on the more classical system, I am quite sure that before being subjected to a steeplechase of secretory phenomena the reader would have benefited from a clear explanation of the role of calcium in secretion from the neuromuscular junction. This would have led naturally to a full discussion of other neurosecretory systems including the adrenal medulla and neurohypophysis and would have provided a sound base for examining systems in which the role of calcium is less clear.

The section entitled 'The role of calcium in secretion' provides a critical survey of current hypotheses. Restricting himself more or less to the classical secretory systems Rubin discusses the various mechanisms that may underlie calcium-dependent exocytosis. The reader cannot fail to be struck by the absence of good experimental evidence on the ultimate mechanism by which calcium effects exocytosis and it is a pity that Dr Rubin does not point more clearly to hopeful new lines of attack.

In the final chapter Rubin discusses the involvement of cyclic AMP in secretion. Although no clear answer emerges, this chapter will be useful to those interested in this vexed question.

Parts of this book are of undoubted interest and will be useful to students of secretion; but the volume as a whole is rather unsatisfactory and its treatment of secretion is neither complete enough nor rigorous enough to ensure that it will not date rapidly.

P. F. Baker

obituary

Max Gluckman, Nuffield Research Professor of Social Anthropology in the University of Manchester, died in Jerusalem on April 13 during the tenure-ship of a visiting professorship at the Hebrew University. He was 64.

Born in Johannesburg, Gluckman began a brilliant academic career at the University of Witwatersrand and went to Exeter College, Oxford in 1934. After being engaged in fieldwork in Zululand from 1936–38, he joined the staff of the Rhodes–Livingstone Institute of Northern Rhodesia. There he embarked on field research in Barotseland, later to form the basis of his researches in anthropological jurisprudence. As director of the Institute from 1941–47, he laid the foundations of what was eventually to become the Manchester School of Theory and Research in Social Anthropology. He expanded the Institute's publication department to reach both a lay and an

academic readership, organising contributions from a wide range of central African social and anthropological problems. He accepted a lectureship at Oxford in 1947, but moved in 1949 to the University of Manchester, where the chair of social anthropology had been specially created for him. He was primarily interested in establishing a research school and began by making his department home-base for his Rhodes–Livingstone team. The main aims of his research workers was to investigate the stresses and strains of modernisation, as well as the tribal custom and law. By the late 1950s the department had extended its research interests to India and the Middle East. He planned extensive and still continuing programmes of collaboration in research and teaching with the Israeli universities, among whose first projects were field studies by Israeli students in Beduin communities. With

his confidence and drive, he organised and dominated the celebrated Gluckman seminar. His circle of contacts and the respect conferred on him by foreign visitors were invaluable links with other fields and views, and his researches led to the publication of *The Judicial Process among the Barotse of Northern Rhodesia*, an analysis of the concept of the 'reasonable man' in African jurisprudence. As chairman of the Association of Social Anthropologists, he organised in 1963 the first Anglo–American conference on new trends in the discipline. Among many academic honours were election as a fellow of the British Academy in 1969, and as an honorary member of the American Academy of Sciences in 1970. Gluckman was a very controversial man and will be remembered for the thought and discussion which he will continue to provoke.

announcements

Award

The **Johann–Georg–Zimmerman Prize** has been awarded to **Peter Magee** for his accomplishments in cancer research.

Appointments

J. W. Murray has been appointed Professor of Geology and head of the department in the University of Exeter.

The title of Central Electricity Generating Board Professor of Corrosion Science has been conferred on **P. R. Swann**.

International meetings

June 5, **Yeasts in industry**, Birmingham (Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS, UK).

June 16–18, **Protein and membrane structure and function**, Honolulu (C. S. McLaughlin, Department of Molecular Biology and Biochemistry, University of California at Irvine, Irvine, California 92664).

Person to Person

Oceanography supervisor. Employed graduate working in London wishing to study for a higher degree in the history of oceanography seeks University Supervisor (Anita McConnell, 11 Arbor Court, Lordship Road, London N16, UK).

Accommodation wanted. British professor, on sabbatical leave from Canada, seeks furnished accommodation somewhere in the UK for his family (10, 7, 4) for the academic year 1975–76. 4 bedrooms, plus study, good-sized garden and easy access to open country essential (Dr A. R. Pugh, Department of Romance Languages, University of New Brunswick, Fredericton, New Brunswick, Canada).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

July 14–18, **Origins and achievements of the Greenwich Observatory 1675–1975**, London (The Secretary, The Organising Committee, 4th Joint IAU/IUHPS Symposium, National Maritime Museum, Greenwich, London SE10 9NF, UK).

July 20–26, **Continuous culture**, Oxford (The Conference Secretariat, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS, UK).

Miscellaneous

Moon mission. NASA is asking for proposals for flight experiments from prospective investigators interested in participating in the Lunar Polar Orbiter mission (June, 1980). The latter is capable of extending to the entire moon, orbital and surface science results obtained from previous lunar missions (especially Apollo), and may serve as a prototype for similar ones to Mars and Mercury. Further information (by June 15, 1975) from: Dr F. D. Martin, Program Manager, Advanced Programs and Technology, Lunar Programs Office, Code SM, NASA, Washington, DC 20546.